NUTRITIONAL ASPECTS OF CANCER

Rosemary Anne Theresa Brennan

A thesis submitted for the degree of

MASTER OF PHILOSOPHY

University of Aston in Birmingham

September 1986

To my family

The University of Aston in Birmingham

Nutritional Aspects of Cancer

by

Rosemary Anne Theresa Brennan

submitted for the degree of Master of Philosophy, September 1986

Various aspects of the complex metabolic syndrome known as cancer cachexia were studied.

The MAC 16 adenocarcinoma of the colon passaged in NMR I mice was found to be a suitable model for the study of cachexia, since it produces a significant weight loss when tumour mass is less than 1% of host body weight without a reduction in food intake.

The effect of the presence of the MAC 16 adenocarcinoma on blood metabolite levels and body composition were also studied. Both the fat and the non-fat mass were reduced in tumour-bearing animals, but despite the loss of adipose tissue there was no evidence of ketosis as might be expected in simple starvation. Both blood glucose and plasma insulin levels were reduced.

The ability of the MAC 16 tumour and other non-involved tissues from NMR I mice to use ketone bodies as an energy source was assessed by measuring levels of the three enzymes required for ketone body utilisation. Low levels of activity of 3-oxo acid-CoA transferase were found in the MAC 16 tumour suggesting that the capacity of the tumour to use ketone bodies as an energy source may be limited.

An attempt was made to reverse cachexia by selectively depriving the tumour of metabolic substrates for energy production by feeding a ketogenic regime, since ketone bodies are thought to maintain homeostasis during starvation. Diets with up to 80% of the energy supplied from medium chain triglycerides, with or without the inclusion of arginine 3-hydroxybutyrate, were fed to NMR I mice. There was a reduction of both host weight loss and tumour size by increasing the lipid contribution of the diet. Under the normal dietary regime the MAC 16 tumour is poorly vascularised and centrally necrotic. After treatment with the ketogenic diets the areas of necrosis were greatly reduced and the degree of vascularisation increased, changes which may make the tumour more vulnerable to chemotherapy and radiotherapy.

Key words: Cancer cachexia
MAC 16 adenocarcinoma
Ketone bodies
3-oxo acid-CoA transferase
vascularisation

Acknowledgements

I would like to thank Dr Michael Tisdale for his support and guidance throughout this research. I also wish to thank Dr Ken Fearon and Dr John Double for their help and co-operation concerning this work. Further thanks are extended to Miss Rebecca Holt, Mrs Andrea Minor and Mr Dave Lambert for their expert technical assistance and to the staff of the animal house for their help.

I acknowledge and thank the Cancer Research Campaign for their financial support of this project.

Finally, I would like to thank Miss Sue Kelly for typing this thesis.

CONTENTS

Page

Section C Methods

Page

Page

Page

List of Figures

Page

Page

Page

Page

Page

Page

Page

Page

Page

Page

Page

List of Tables

Page

Page

Page

Page

Page

Abbreviations

AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
BCAA	branched chain amino acids
CoA	coenzyme A
DTNB	5'5'-dithiobis(2'-nitrobenzoic acid)
EDTA	ethylenediaminetetraacetic acid
FA	fatty acid
FAD	flavin adenine dinucleotide
$FADH_2$	flavin adenine dinucleotide, reduced form
FFA	free fatty acid
KB	ketone body
MCT	medium chain triglyceride
MEHA	3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced form
3OHB	3-hydroxybutyrate
PDH	pyruvate dehydrogenase
PPi	inorganic pyrophosphate
S.E.M.	standard error of the mean
s.c.	subcutaneously
TCA	tricarboxylic acid cycle
TG	triglyceride
TNF	tumour necrosis factor
U.V.	ultraviolet
VLDL	very low density lipoproteins

A. Introduction

A.1 Nutrition and Cancer

The nutritional status of the host may be seriously affected by the presence of cancer. The type and site of the cancer may be a major contributory factor towards the weight loss and malnutrition seen in many cancer patients. Tumours of the oral cavity and gastro-intestinal tract, for example, may prevent either the ingestion or digestion of food. Progressive weight loss and malnutrition may also occur without the involvement of the gastrointestinal tract or other vital organs and with tumours whose total mass is only a small fraction of total body weight. These are distinguishing features of a condition known as cachexia which is frequently found in patients with neoplastic diseases.

Surgery, chemotherapy and radiotherapy used in the treatment of cancer are also known to affect adversely the nutritional status of the host. Chemotherapeutic agents and radiotherapy are active on rapidly proliferating cells and therefore it is not surprising that the mucosa of the gastrointestinal tract, which has cells with a rapid growth fraction, is seriously affected, resulting in malabsorption of nutrients. Methotrexate and 5-fluorouracil are two chemotherapeutic agents well known for their toxicity on the gastro-intestinal tract mucosa (Greene et al, 1979). Nausea, vomiting, altered taste sensations and learned food aversions are also associated with the use of radiotherapy and chemotherapeutic agents and arise as a result of the functional disturbance of some central autonomic control, possibly the emetic centre of the lateral reticular formation of the medulla in the brain (Morrison, 1979). If a patient receiving such treatment is already in a debilitated state then this situation may be further aggravated and lead to a poor

prognosis for recovery. Enteral and parenteral hyperalimentation have been used extensively in an attempt to reverse the debilitating effects of the disease and antineoplastic therapy, but results so far have been disappointing, host reserves only being partially restored (Nixon et al, 1981).

Tumours require certain nutrients for growth, which are acquired at the expense of the host, and a thorough understanding of host and tumour nutrition is therefore very important so that any nutritional support given to the host does not actually promote tumour growth. It might also be possible to exploit any differences which exist between host and tumour nutrition to reduce tumour growth and, at the same time, improve the nutritional status of the host. Such strategies have been considered particularly in the treatment of cancer cachexia (Conyers et al, 1979a; 1979b; Williams and Matthaei, 1981; Tisdale and Brennan, 1983; Fearon et al, 1985).

A.2 Cancer and Cachexia

Cachexia is a frequent accompaniment of neoplastic diseases and has long been recognised as one of the major causes of death in the cancer patient, accounting for between 20-60% of all deaths (Harnet, 1952; Inagaki et al, 1974; Warnold et al, 1978). It was thought to arise as a result of a progressively inadequate dietary intake, but since many patients present initially with a significant weight loss as their first and only symptom, despite a completely adequate dietary intake, it is obviously more complex than this. Coupled with the observation that cancer cells generally have been shown to exhibit high rates of anaerobic glycolysis (Gold, 1974), which is considered to be an outstanding metabolic characteristic of these

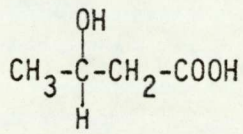
type of cells, it suggests the existence of a specific mechanism peculiar to the cancerous condition by which weight loss and body debilitation can proceed.

Cancer cachexia has been described as a metabolic syndrome characterised by anorexia, asthenia, anaemia, an increased metabolic rate and increased energy expenditure with progressive weight loss resulting from significant losses of body fat, protein and other body components (Costa, 1977; Bozzetti, 1984). The precise mechanism by which a tumour produces the cachectic syndrome has yet to be fully determined, although a number of explanations regarding its pathogenesis have been proposed (Gold, 1974; Stein, 1978; Theologides, 1979; Williams and Matthaei, 1981; Lazo, 1985).

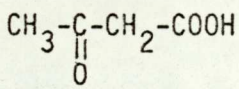
A.3 Cachexia and Starvation

Although cachexia is similar in respect to weight loss to starvation, there are important differences which exist between the two conditions, particularly in relation to metabolism. Starvation is a hypometabolic state in which there is a reduction in the turnover of carbohydrate, fat and protein stores. This reduction in utilisation of nutrient stores is regulated by ketone bodies, 3-hydroxybutyrate and acetoacetate, the structures of which are shown in figure A.3.1. The sources of fuel in starvation are shown in figure A.3.2.

Following the onset of starvation, free fatty acids are mobilised from adipose tissue as a result of a fall in plasma insulin levels and a rise in glucagon levels and excess free fatty acids are converted to ketone bodies in the liver. Part of the adaptation by the body to starvation is the ability of the brain and other tissues



3-Hydroxybutyrate



Acetoacetate

Figure A.3.1 Chemical structures of ketone bodies

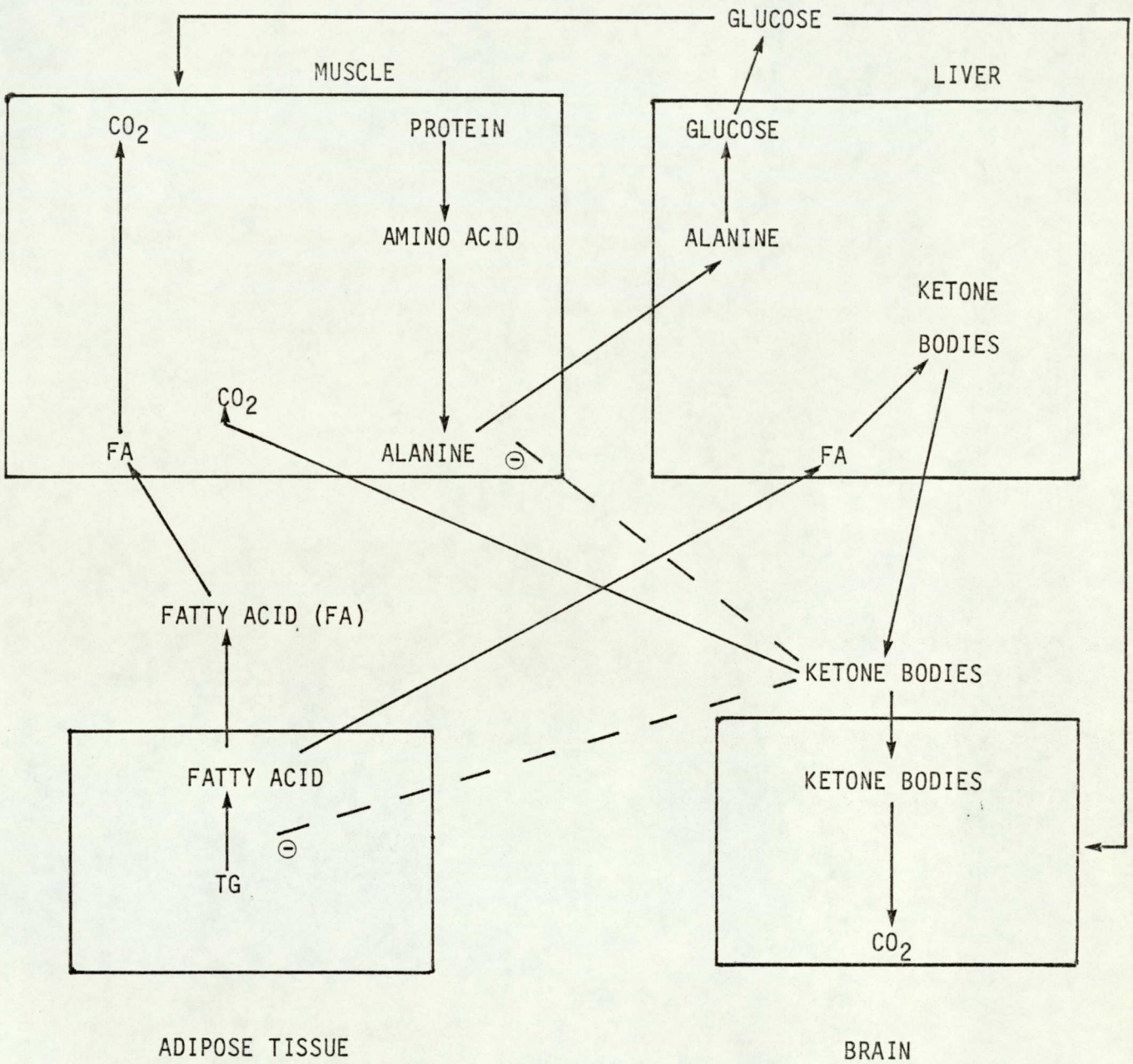


Figure A.3.2 Fuel sources under normal starvation conditions

to use ketone bodies as the predominant metabolic fuel in place of glucose (Owen et al, 1967) and so conserve muscle which may be broken down via the various metabolic pathways to form glucose. High levels of ketone bodies stimulate the release of insulin from the B-cells of the pancreas, and since insulin is a potent anti-lipolytic hormone, this decreases the rate of free fatty acid mobilisation from adipose tissue. There is also evidence that high ketone body levels can directly reduce lipolysis (Hawkins et al, 1971). Thus, the rate of ketone body consumption can, to some extent, regulate their own production. A flow cycle of the metabolism of ketone bodies is shown in figure A.3.3.

As well as providing an alternative source of metabolic fuel to glucose, the metabolism of ketone bodies and fatty acids inhibits glucose oxidation in extrahepatic tissues. Rapid oxidation of ketone bodies and fatty acids results in the increased acetylation of coenzyme A, and this in turn leads to an increase in the proportion of the inactive form of pyruvate dehydrogenase (figure A.3.4), thus preventing irreversible loss of glucose carbon. The oxidation of pyruvate to acetyl-CoA is irreversible and represents a loss to body carbohydrate reserves because animal cells lack the capacity for the synthesis of glucose from acetyl-CoA. Regulation of this step is therefore essential. Pyruvate dehydrogenase exists in a phosphorylated (inactive) and dephosphorylated (active) form, and these are interconvertible by a phosphatase and a kinase. Insulin and raised cell Ca^{2+} levels activate the phosphatase resulting in increased pyruvate dehydrogenase activity. The kinase, on the other hand, is activated by increased levels of acetyl-CoA and NADH which leads to the inhibition of pyruvate oxidation. Any glycolytic break-down of glucose would thus be channelled to lactate and can be

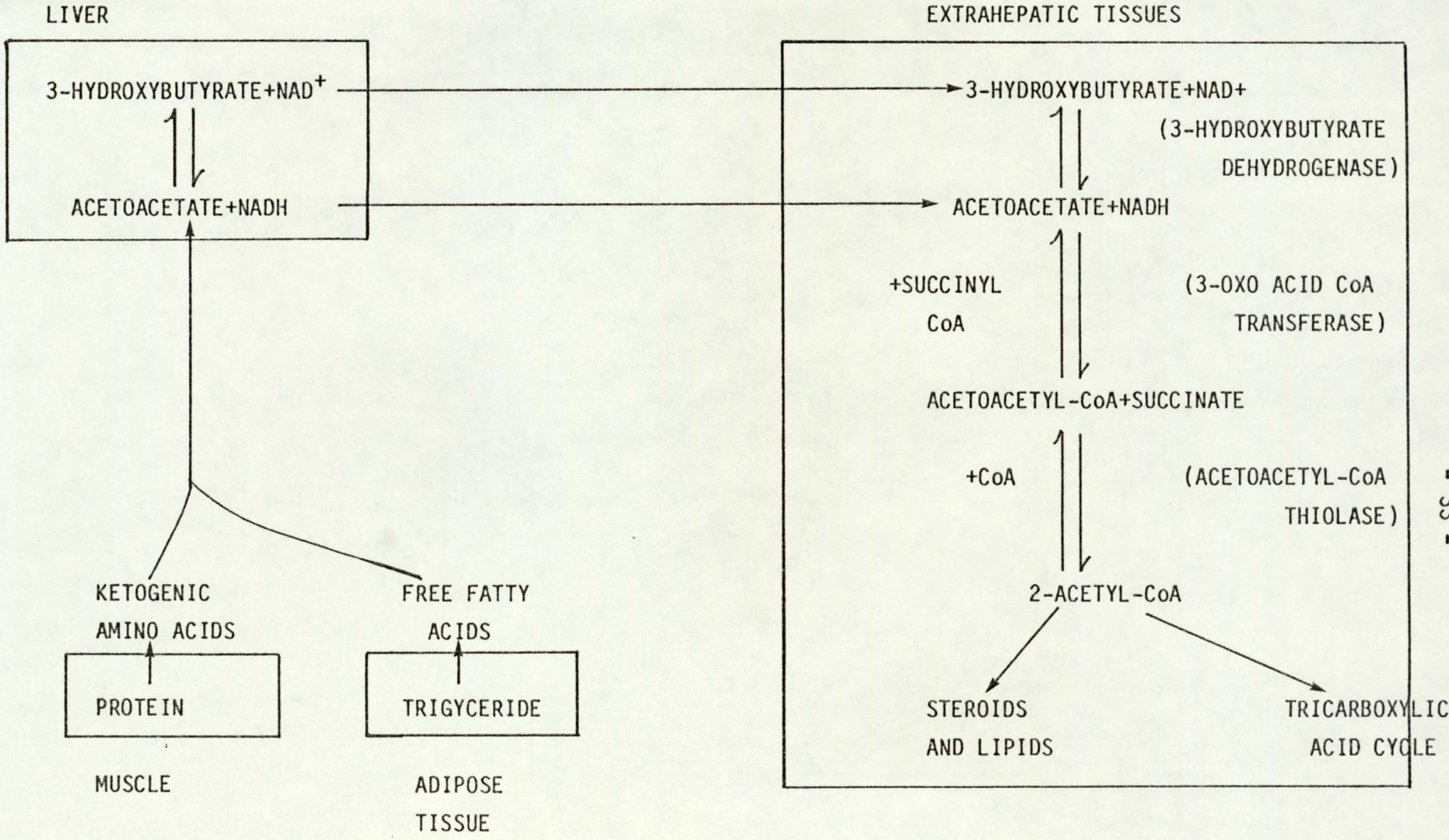


Figure A.3.3 Ketone Body Metabolism

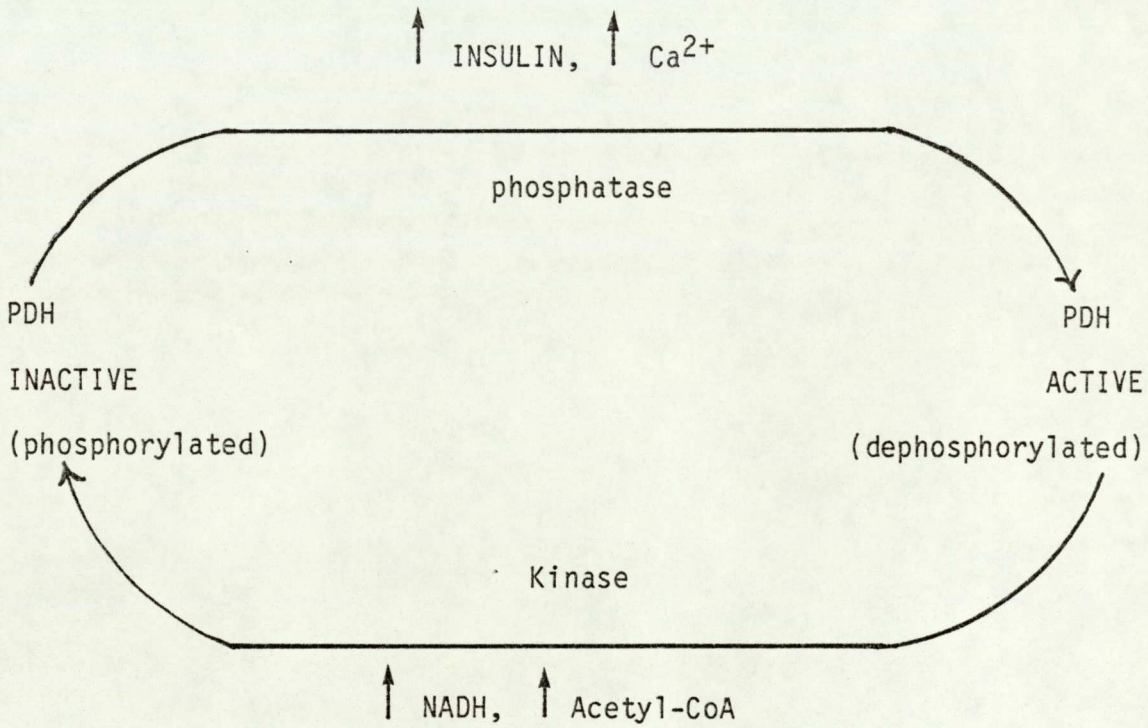


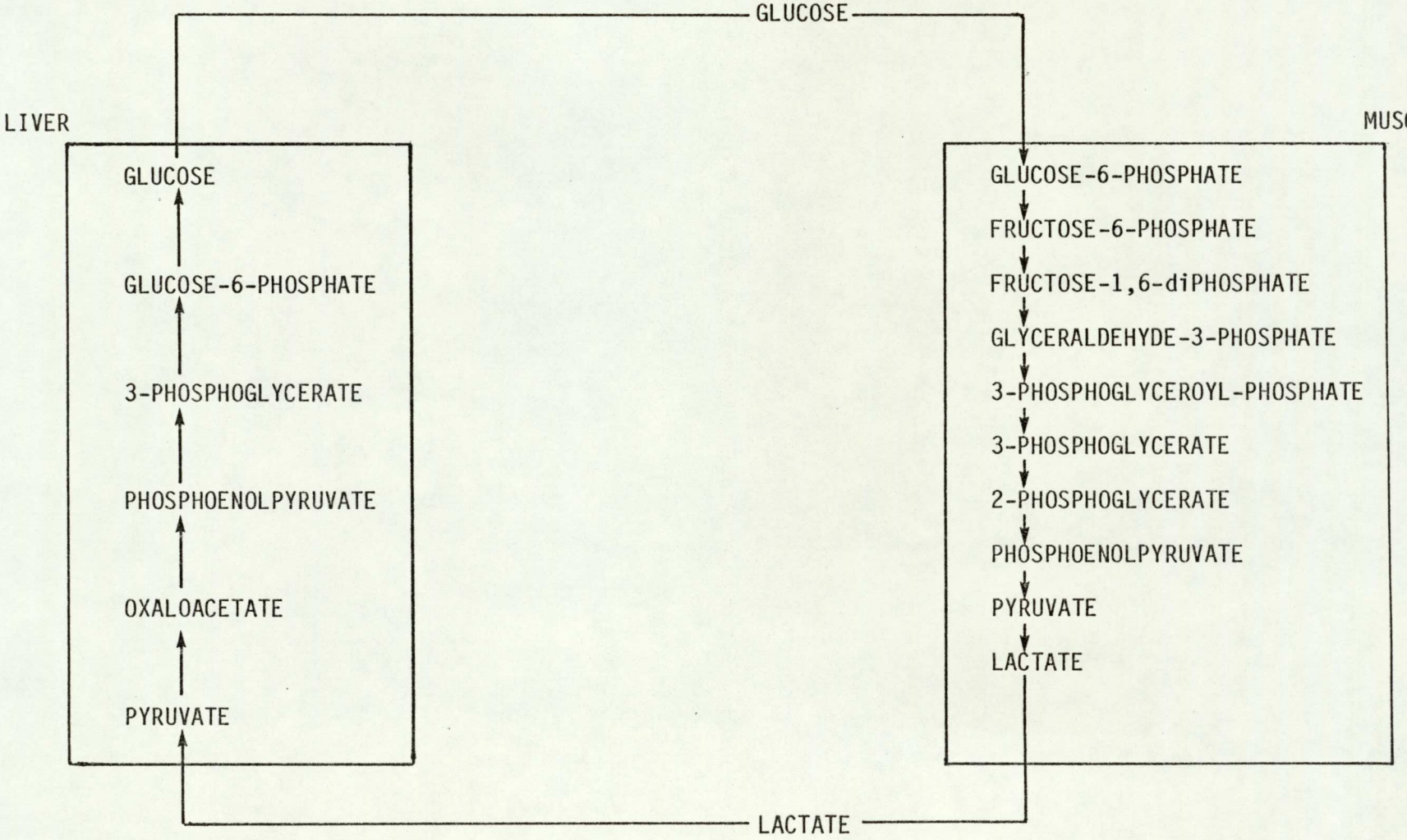
Figure A.3.4 Pyruvate dehydrogenase control

converted to glucose again by hepatic gluconeogenesis. Thus, in starvation, the Cori cycle (figure A.3.5) driven by fatty acid oxidation in the liver is maintained by the glucose sparing effect of fatty acid and ketone body oxidation in muscle. These actions of fatty acids and ketone bodies resulting in the sparing of glucose for brain metabolism during the early stages of starvation are shown in figure A.3.6.

With prolonged starvation, two metabolic changes occur which are essential to survival. The first is the oxidation of ketone bodies by the brain and the second is the inhibition of protein breakdown. Glucose uptake by the brain is decreased as a result of high ketone body levels in the blood and as a consequence of this the brain switches to using ketone bodies as the predominant metabolic fuel. Ketone bodies also have an inhibitory effect on the release of alanine from muscle and so plasma alanine levels fall and there is also a reduction in urinary nitrogen excretion apparently reflecting a reduction in protein breakdown (Sherwin et al, 1975). The oxidation of branched-chain amino acids is also inhibited by high levels of ketone bodies (Buse et al, 1972). Since these amino acids are the major source of α-amino nitrogen for the synthesis of alanine and glutamine (Adibi, 1984) and are themselves known to inhibit protein catabolism in muscle, their accumulation could also mediate the observed inhibition of protein breakdown (figure A.3.7). It appears, therefore, that ketosis is an important feature of starvation.

In contrast, cancer cachexia is a hypermetabolic state with increases in glycolysis, gluconeogenesis, lipolysis and catabolism, all of which are inappropriate to the weight losing state, especially as they tend to increase energy expenditure and loss of fat and non-

Figure A.3.5 THE CORI CYCLE



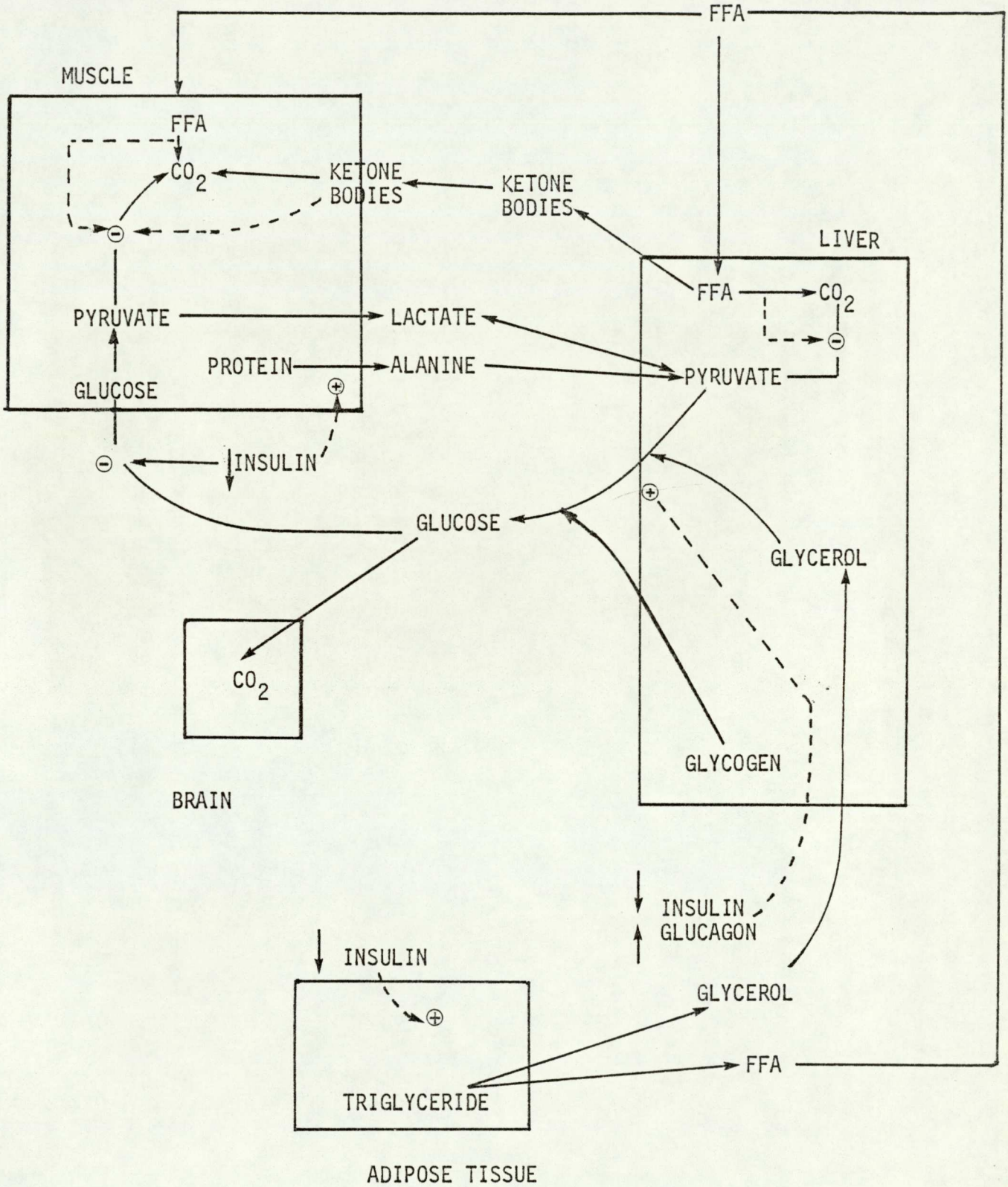


Figure A.3.6 Glucose sparing for use by the brain in the early phase of starvation

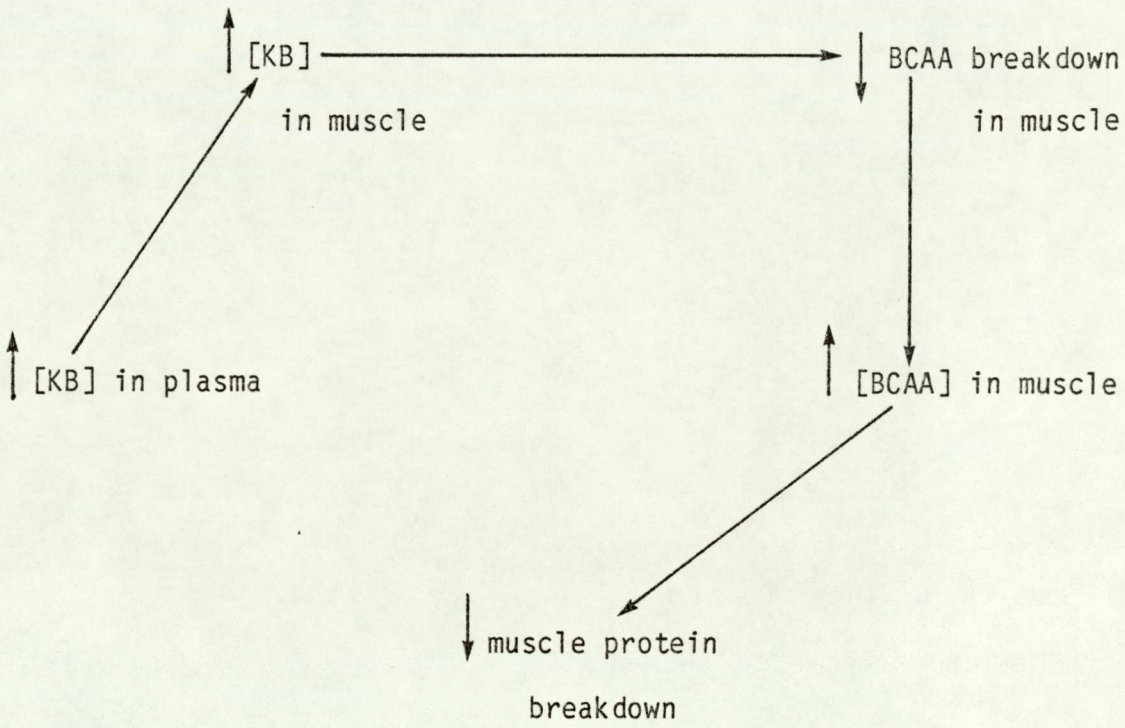


Figure A.3.7 Inhibition of protein breakdown in muscle.

BCAA, Branched chain amino acids

KB, Ketone bodies

fat mass. The sources of fuel in the cachectic cancer patient are shown in figure A.3.8. One reason for increased energy expenditure in cancer patients may be due to increased Cori cycle activity; that is, the conversion of lactate to glucose in the liver which has been found to be high in patients with metastatic carcinoma and progressive weight loss (Holroyde et al, 1975). Gluconeogenesis from lactate is a particularly inefficient process consuming 6 molecules of ATP per futile Cori lactate-glucose cycle, and as the tumour enlarges consuming ever increasing amounts of glucose, the energy resources of the host become increasingly depleted by the futile efforts to maintain normal blood glucose levels (Gold, 1974).

The most obvious sign of protein deficiency in the cachectic cancer patient is the loss of skeletal muscle mass which may arise as a result of increased protein degradation and decreased protein synthesis. In studies using tumour-bearing rats it was found that the total protein content of muscle was significantly decreased and the incorporation of labelled amino acids into gastrocnemius muscle protein markedly inhibited (Clark et al, 1971). Rates of whole body protein turnover were shown to be higher in cancer patients compared to non-cancer patients and normal fasting subjects (Jeevanandam et al, 1984); an increased rate of muscle catabolism and glucose production have also been observed in lung cancer patients. As a result of increased protein degradation in muscle, plasma amino acid levels are increased, and since tumour cells have the ability to concentrate amino acids from the plasma amino acid pool more efficiently than normal cells (Wiseman and Ghadially, 1955; Copeland et al, 1979), the tumour acts as a nitrogen trap producing a negative nitrogen balance in the host.

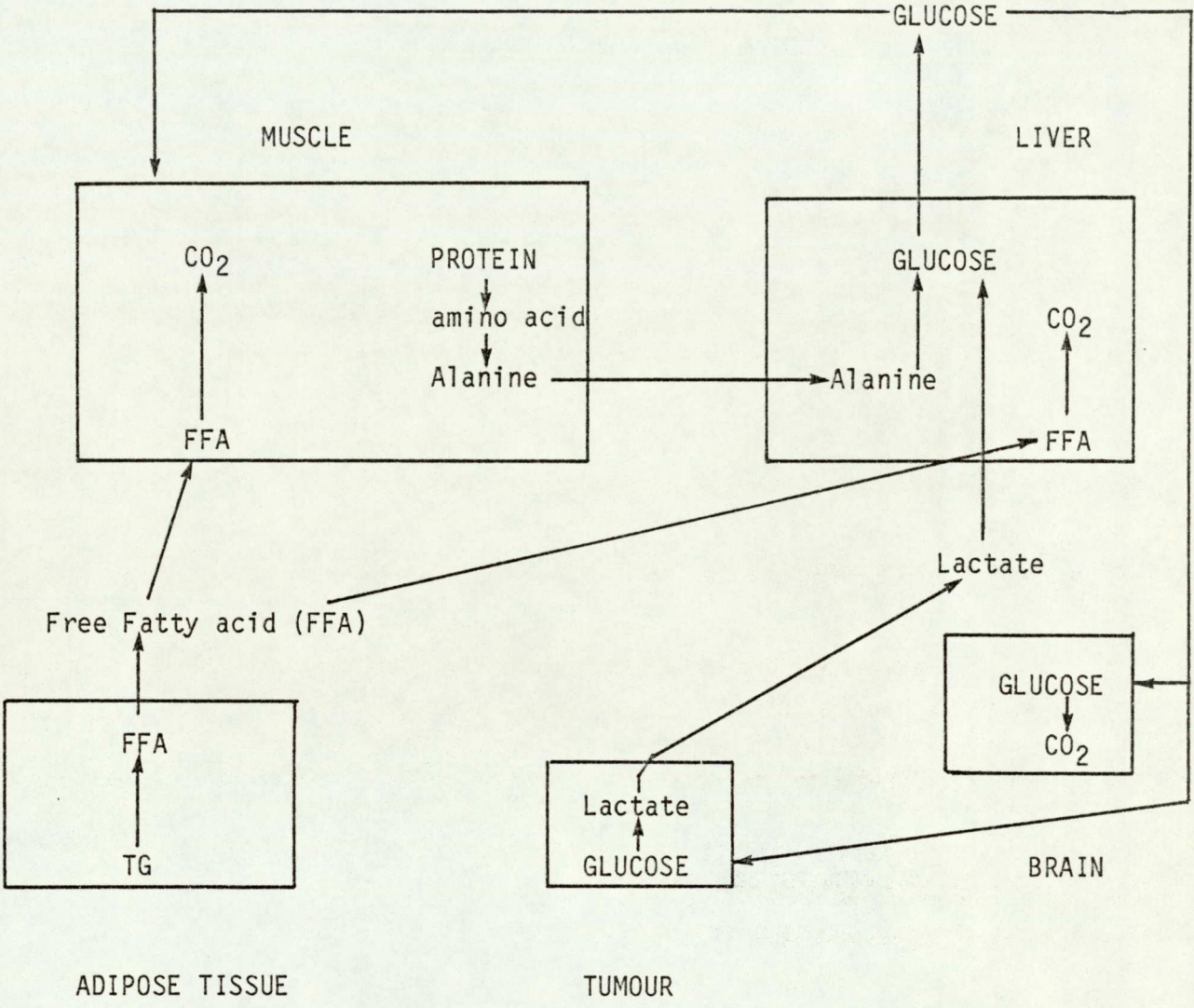


Figure A.3.8 Fuel sources in the cachectic cancer patient

TG, Triglyceride

FFA, Free fatty acid

Mider et al (1949) found that the Walker 256 carcinoma growing in young rats contained between 20-40 per cent of the animal's total body nitrogen at death. The tumour is able to utilise the amino acids for protein synthesis. Oxidation of branched-chain amino acids is also increased in cachexia resulting in raised plasma alanine levels (Williams and Matthaei, 1981) and this alanine may then be utilised in gluconeogenesis in the liver. In patients with progressive malignant disease the conversion of alanine to glucose was found to be increased in the overnight fasting state compared with a control group without malignant disease (Waterhouse et al, 1979).

Profound alterations of the lipid metabolism of tumour-bearing animals and humans have also been reported by several investigators (Mider et al, 1949; Watkin, 1959; Costa and Holland, 1962; Kralovic et al, 1979), with the final result being the depletion of body fat. Loss of body lipid in cancer results from a primary effect on the mobilisation of free fatty acids from adipose tissue in the host and this mobilisation may occur early on in the development of the tumour. It may occur when the tumour mass is very small and is associated with increased plasma lipid concentrations, changes in the composition of plasma lipoproteins and also a decrease in the activity of lipoprotein lipase, the key enzyme required for triacylglycerol clearance from the blood. Increased concentrations of very low density lipoprotein (VLDL) triacylglycerols have been found in the plasma of tumour-bearing rats (Redgrave et al, 1984) along with low levels of glucose and insulin (Devereux et al, 1982; Lanza-Jacoby et al, 1982). Low plasma insulin levels would impair the function of lipoprotein lipase in addition to promoting free fatty acid release

from adipose tissue. These effects would favour expansion of the plasma triacylglycerol pool through increased VLDL production rates and decreased efficiency of lipoprotein lipase. Therefore, it is possible that the increase in free fatty acid mobilisation observed in tumour bearing subjects is related to a decrease in plasma insulin levels.

The mechanism by which the tumour leads to depletion of the host's body fat and hyperlipidemia is unknown. The tumour may have a primary effect on insulin synthesis or secretion which in turn would alter lipid metabolism, alternatively it has been postulated that tumour cells may produce or stimulate the host to produce a fat mobilising factor (Mays, 1969; Kitada et al, 1980). Frederick and Begg (1956) observed that when tumours were removed surgically from tumour bearing animals, the cachectic process was reversed. A lipolytic substance, toxohormone L, has been isolated from the ascites fluid of S180 sarcoma cells and has also been found in the ascites fluid of pateints with hepatoma and Grawitz's tumour (Masuno et al, 1981). More recently, studies in which the effects of infection induced cachexia in rabbits were studied have led to the isolation of a macrophage factor termed cachectin, which has been found to suppress the activity of the enzyme lipoprotein lipase both in vivo and in vitro. Suppression of this enzyme leads to a hypertriglyceridaemic state in the affected animals (Beutler et al, 1985a), and it has been suggested that cachectin may contribute to the lipid and protein catabolism that ultimately reduces the host to the cachectic state. Furthermore, a similarity in structure has been found between cachectin and tumour necrosis factor (TNF), another macrophage product (Beutler et al, 1985b).

Metabolic utilisation by the tumour of plasma free fatty acids

has been shown to be less than one per cent of total flux (Mermier and Baker, 1974) and no differences were found in the oxidation of injected radiolabelled VLDL to carbon dioxide between groups of tumour-bearing and non tumour-bearing mice (Ookhtens and Baker, 1979). These results exclude major utilisation of lipids as an oxidative substrate by the tumour. Some tumours are unable to utilise fat as a metabolic substrate due to a deficiency in some of the enzymes required for its oxidation. Hepatoma 7777, for example, was shown to exhibit low levels of fatty acyl coenzyme A, and a virtual absence of carnitine palmitoyl transferase activity, and fortified homogenates of the tumour were unable to oxidise palmitate (Fields et al, 1981). A flow diagram of fatty acid oxidation is shown in figure A.3.9. Slowly growing tumours which lack the capacity for glucose phosphorylation have been shown to oxidise fatty acids readily, while tumours which exhibit high rates of glycolysis are unable to oxidise fatty acids (Bloch-Frankenthahl et al, 1965). The other tissues of the host are able to utilise fatty acids readily as a source of energy and are therefore likely to be the major consumers of this source of metabolic fuel.

Ketosis is an uncommon phenomenon in cancer patients (Conyers et al, 1979a) and the absence of ketosis may account for some of the metabolic abnormalities seen in cachectic cancer patients. Furthermore, Conyers and others (1979a) have proposed that iatrogenically induced ketosis may provide a physiological means of regulating host glucose metabolism with a consequent reduction in host weight loss and reversal of the cachectic process. Dietary induced systemic ketosis has been shown to reduce blood glucose levels and glucose utilisation in man (Phinney et al, 1983). Tumours, especially those with a poor blood supply, might be expected

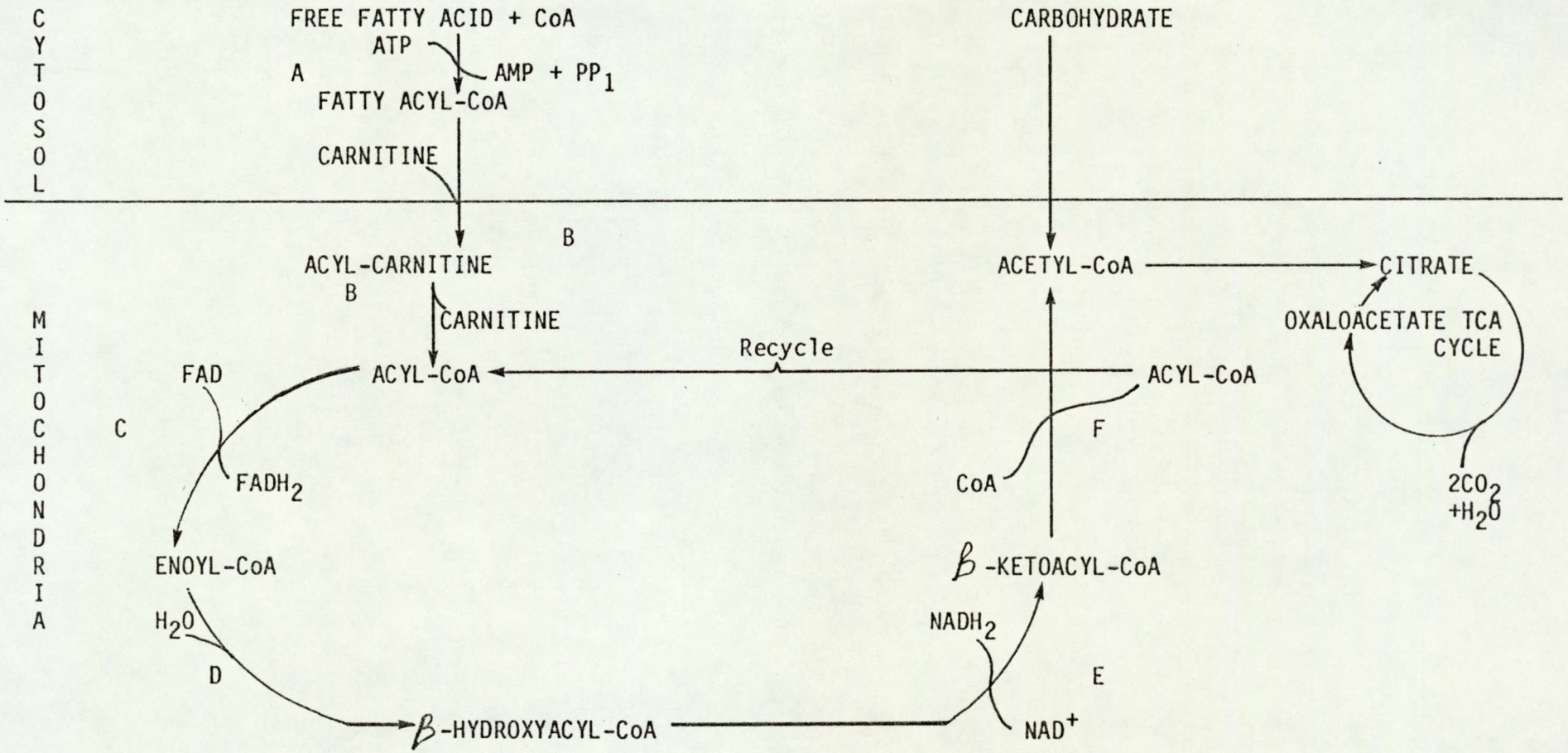


Figure A.3.9 β **-OXIDATION CYCLE**

A - THIOKINASE, B - CARNITINE ACYLTRANSFERASE, C - ACYL-CoA DEHYDROGENASE, D - ENOL HYDRATASE, E - 3-HYDROXYACYL DEHYDROGENASE, F - THIOLASE

to use glucose primarily as an energy source since the Embden-Meyerhof pathway is the only means of ATP production which does not require oxygen. Thus a low carbohydrate high fat ketogenic diet might be expected to provide little nourishment for the tumour since the precursors for gluconeogenesis other than lactate would be reduced.

The utilisation of ketone bodies by the tissues requires the presence of three enzymes, 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), 3-oxo acid-CoA transferase (EC 2.8.3.5), and acetoacetyl-CoA thiolase (EC 2.3.1.9). The three reactions involved in the conversion of ketone bodies to acetyl-CoA are shown in figure A.3.10. Many tumours would be expected to be unable to utilise ketone bodies as a metabolic fuel, since levels of 3-oxo acid CoA transferase have been found to be either decreased or absent in a range of murine (Fields et al, 1981; Tisdale and Brennan, 1983) and human tumours (Frederick and Ramsey, 1978). Metabolism of ketone bodies to produce ATP also requires oxygen and this would be reduced in poorly vascularised tumours. Ketosis may be one method, therefore, with which to provide a relatively host specific substrate and therapeutically starve the tumour.

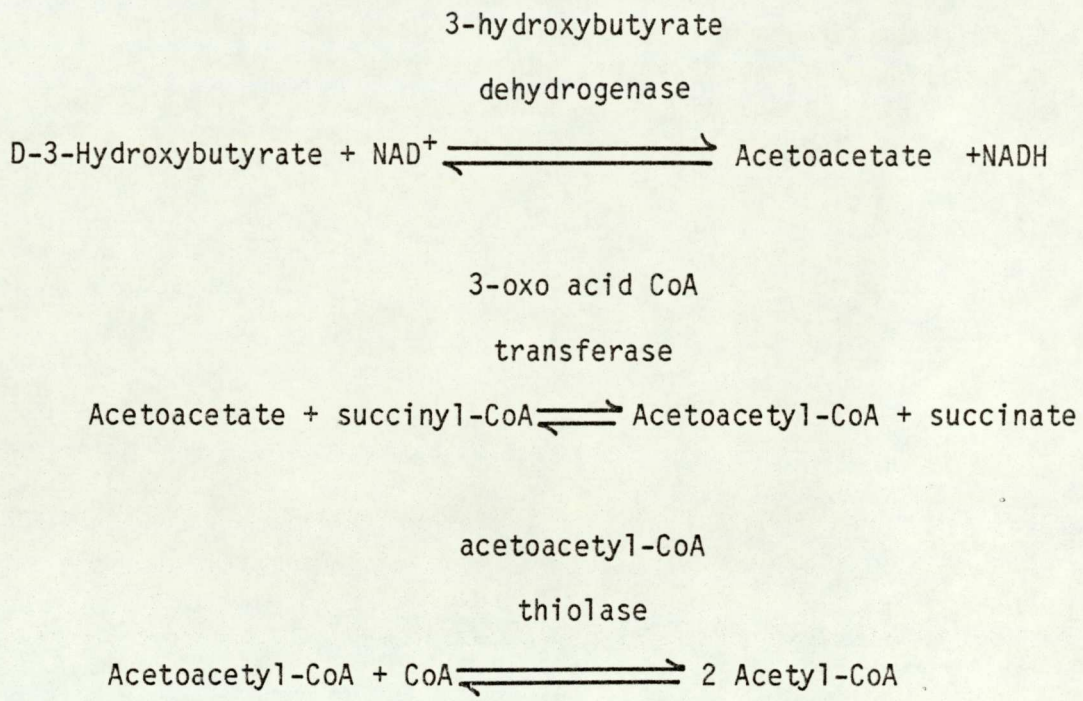
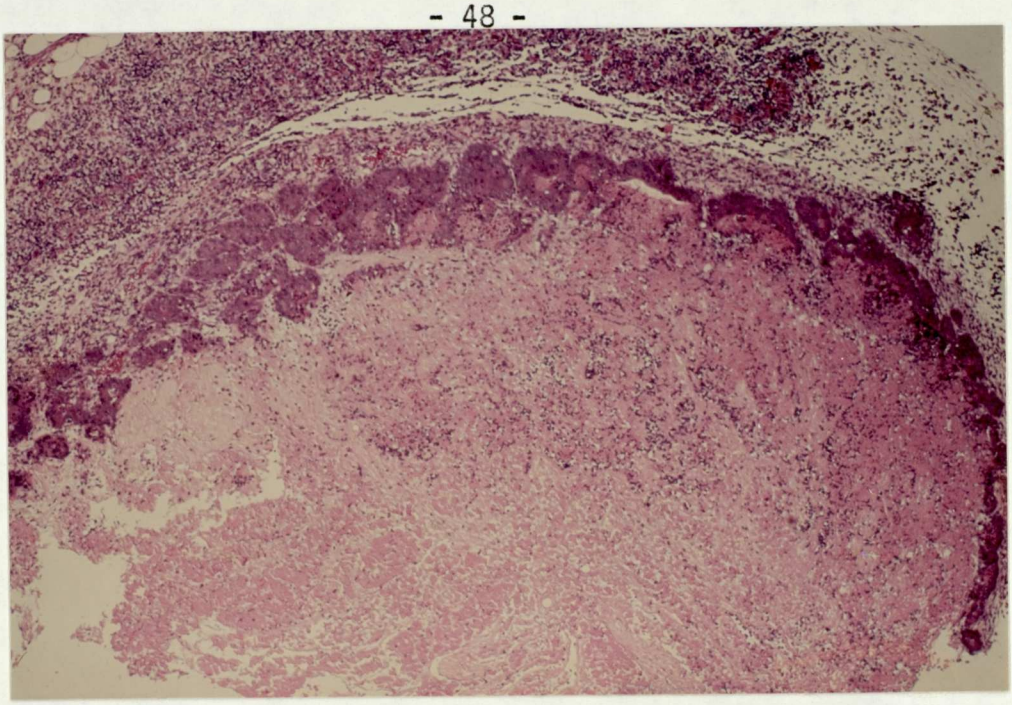


Figure A.3.10 The reactions involved in the utilisation of ketone bodies

A.4 Aims and scope of the present investigation

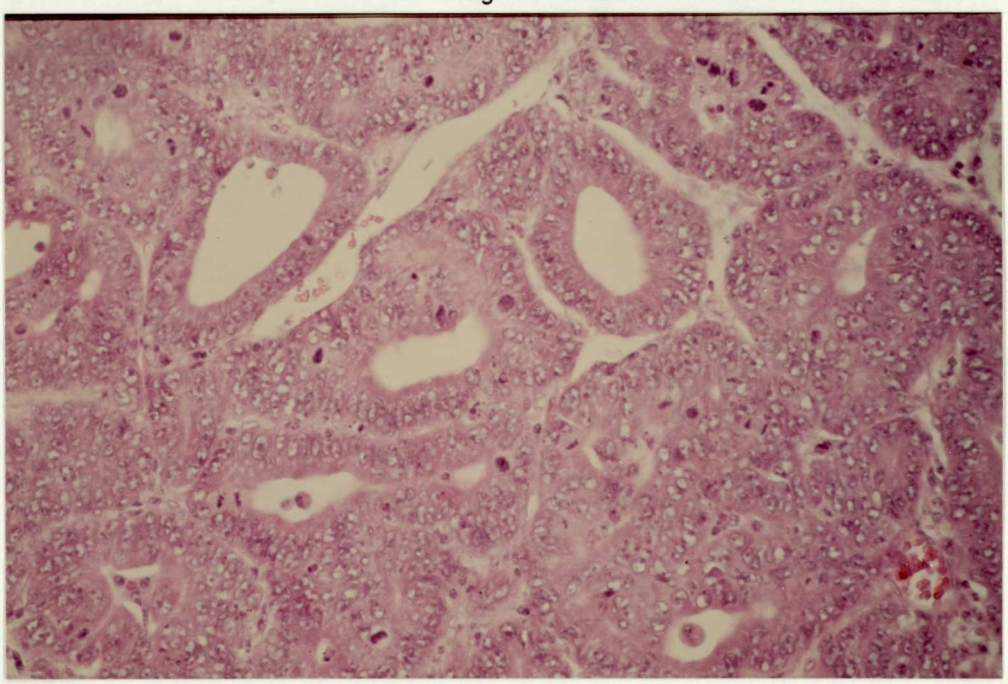
The various aspects of cachexia described in sections A.2 - A.3 were investigated in an experimental model of cachexia which utilises the MAC 16 adenocarcinoma of the colon transplanted in NMR I mice. The MAC 16 tumour, which is one of a series of colonic tumours originally induced in NMR I mice by 1,2-dimethylhydrazine (Double et al, 1975), is moderately well-differentiated (figure A.4.1) and produces extensive weight loss in the host without a concurrent reduction in food intake (Ali et al, 1985). The aims of the study were, firstly, to confirm that the MAC 16 tumour is a suitable model of cachexia by monitoring body weight, body composition and tumour growth after implantation of the tumour into NMR I mice. Secondly, to measure levels of the three enzymes required for the utilisation of ketone bodies, 3-hydroxybutyrate dehydrogenase, 3-oxo acid CoA transferase and acetoacetyl-CoA thiolase in the tissues of NMR I mice to see whether the presence of the MAC 16 tumour has any effect on these levels, and therefore on the ability of the tissues to utilise ketone bodies as a source of metabolic fuel, including the tumour itself. Thirdly, to measure levels of a number of metabolites in the blood of NMR I mice in order to see what effect tumour growth has on these levels. Fourthly, to study the effect of different diets, normal, high carbohydrate and high fat on tumour growth, body weight, body composition and metabolite levels in NMR I mice. The overall aim of the investigation was to provide an insight into the aetiology and consequences of cachexia using the MAC 16 tumour model, and to investigate the possibility of reversing the effects of cachexia using dietary means.

A)



x10 magnification

B)



x200 magnification

Figure A.4.1 Sections through the MAC 16 adenocarcinoma from NMR I mice.

A) x10 magnification. The tumour is centrally necrotic and poorly vascularised

B) Higher magnification (x200). The tumour displays an acinar structure typical of colonic epithelium.

B. Materials

B.1 Biochemicals

The following biochemicals were purchased from the sources indicated.

B.1.1 Alpha Laboratories Ltd (Hampshire, UK)

Wako NEFAc kit for the determination of plasma free fatty acids.

B.1.2 BDH Chemicals Ltd (Dorset, UK)

Ethylene diaminetetra-acetic acid, disodium salt AR.

Folin and Ciocalteu phenol reagent

Glycine AR.

Iodoacetamide.

B.1.3 Sigma Chemical Co Ltd (Poole, Dorset, UK)

Acetoacetic acid, lithium salt.

Acetoacetyl-coenzyme A, sodium salt.

Acetyl-coenzyme A, sodium salt.

Adenosine-5'-triphosphate, trisodium salt.

Bovine serum albumin.

Carnitine acetyltransferase, from pigeon breast muscle.

Coenzyme-A, sodium salt.

5'5'-Dithiobis(2'-nitrobenzoic acid) DTNB.

Glycerokinase, from candida mycoderma.

α-Glycerophosphate dehydrogenase, Type 1 from rabbit muscle.

Heparin.

Hydrazine hydrate.

β-Hydroxbutyrate dehydrogenase, Type II from Rhodopseudomonas spheroides.

L-lactic dehydrogenase, Type II from rabbit muscle.

2-Mercaptoethanol.

Nicotinamide adenine dinucleotide, (NAD) Grade IV.

Nicotinamide adenine dinucleotide, reduced form (NADH) Grade III.

Succinyl-coenzyme A, sodium salt.

O-Toluidine reagent kit, for the determination of glucose in plasma, serum and whole blood.

Triethanolamine hydrochloride.

Trizma base.

B.2 Chemicals

The following compounds were purchased from the sources indicated.

B.2.1 BDH Chemicals Ltd (Dorset, UK)

Cupric sulphate, anhydrous AR.

Magnesium chloride, hexahydrate AR.

Perchloric acid, 73% w/v AR.

Potassium chloride AR.

di-Potassium hydrogen orthophosphate, trihydrate AR.

Potassium dihydrogen orthophosphate, anhydrous AR.

Sodium potassium tartrate AR.

B.2.2 Fisons Scientific Equipment (Loughborough, Leics, UK)

Hydrochloric acid 36%

Potassium hydroxide

Sodium carbonate, anhydrous

Sodium hydroxide

B.3 Solvents

The following solvents were purchased from the sources indicated.

B.3.1 Burroughs Ltd (London, UK)

Absolute ethanol

B.3.2 May & Baker Ltd (Dagenham, UK)

Chloroform

Diethyl ether

B.3.3 Tennants Ltd (West Bromwich, West Midlands, UK)

Acetone

Methanol

B.4 Gases and agents for anaesthesia

The following medical gases were purchased from BOC Ltd (London, UK).

Nitrous oxide

Oxygen

Fluothane inhalation anaesthetic was obtained from ICI (Macclesfield, Cheshire, UK).

B.5 Diet Components

Diet components were purchased from the sources indicated.

B.5.1 **BDH Chemicals Ltd (Dorset, UK)**

Sodium chloride AR

Sucrose AR

B.5.2 **Pilsbury's Ltd (Birmingham, West Midlands, UK)**

Dicalcium phosphate

Rat and mouse breeding diet 41B

Rodent 006 premix

Sodium caseinate

Soya

B.5.3 **Scientific Hospital Supplies Ltd (Liverpool, UK)**

Liquigen medium triglyceride emulsion, containing medium triglyceride 52%, water 48%, calorific value 1.7 MJ% (400 KCal)

B.5.4 **Sigma Chemical Co Ltd (Dorset, UK)**

Bentonite

Calcium carbonate

L-Methionine

B.5.5 **Gifts**

3-Hydroxybutyrate, arginine salt was kindly donated by Solvay et Cie (Brussels).

B.6 **Animals, maintenance of tumour and diet**

Pure strain inbred NMR I mice (8-10 weeks) were kindly supplied by Dr K H Fearon, Department of Medical Oncology, University of

Glasgow and Dr J A Double, Clinical Oncology Unit, University of Bradford. Animals implanted with and without MAC 16 tumour were supplied.

B.6.1 Transplantation of tumour

MAC 16 tumour was excised from donor animals, placed in sterile 0.9% saline and cut into small fragments 1x2 mm in size. Fragments were then implanted subcutaneously into the flank of the right hind limb using a trocar.

B.6.2 Feeding

Mice were fed on diet 8b (James Burill and Sons, Cleckheaton, UK) prior to arrival and thereafter on rat and mouse breeding diet and water ad-libitum unless otherwise stated. Tumour-bearing and non tumour-bearing mice were housed in separate rooms to eliminate the possibility of interference from pheromones on the results of the studies.

B.7 Buffer solutions

B.7.1 0.01M Tris-HCl Buffer pH 7.4 containing 0.25M sucrose and 1 mM 2-mercaptoethanol

Trizma base	1.21 g
Sucrose	85.57 g
2-Mercaptoethanol	0.078 g
Distilled water	900 ml

The pH was adjusted to 7.4 with concentrated hydrochloric acid and the final volume was made up to 1 litre with distilled water.

B.7.2 **0.1M Tris-HCl Buffer pH 8.5**

Trizma base	6.05 g
Distilled water	400 ml

The pH was adjusted to 8.5 with concentrated hydrochloric acid, and the final volume was made up to 500 ml with distilled water.

B.7.3 **0.05M Tris-HCl Buffer pH 8.5**

Trizma base	3.025 g
Distilled water	400 ml

The pH was adjusted to 8.5 with concentrated hydrochloric acid, and the final volume was made up to 500 ml with distilled water.

B.7.4 **Hydrazine Tris Buffer pH 8.5**

Hydrazine hydrate	2 ml
0.1M Tris-HCl buffer pH 8.5	40 ml

The pH was adjusted to 8.5 with concentrated hydrochloric acid, and the final volume was made up to 50 ml with 0.1M Tris HCl buffer pH 8.5.

B.7.5 **Hydrazine-Glycine Buffer pH 9.0 (0.4 M hydrazine, 0.5 M glycine)**

Hydrazine hydrate	25 ml
Glycine	11.4 g
Distilled water	200 ml

The pH was adjusted to 9 with concentrated hydrochloric acid, and the final volume was made up to 300 ml with distilled water.

B.7.6 **Hydrazine-Glycine Buffer pH 9.8 (1 M hydrazine, 0.2 M glycine) containing 2 mM magnesium chloride**

Hydrazine hydrate	20.8 g
Glycine	1.5 g
1 M Magnesium chloride	0.2 ml
Distilled water	50 ml

The pH was adjusted to 9.8 with a few drops of 10 M KOH, and the final volume was made up to 100 ml with distilled water.

B.7.7 **0.1 M Potassium phosphate buffer pH 6.8**

Solution A

Potassium dihydrogen orthophosphate 13.609 g made up to 1 litre with distilled water.

Solution B

di-Potassium hydrogen orthophosphate, trihydrate 22.82 g made up to 1 litre with distilled water.

Solution B was added to 200 ml of solution A until a pH of 6.8 was reached.

B.7.8 **0.5 M Triethanolamine Buffer pH 7.6, containing 5 mM EDTA**

Triethanolamine hydrochloride	23.3 g
EDTA, disodium salt	0.47 g
Distilled water	200 ml

The pH was adjusted to 7.6 with 2M NaOH, and the final volume made up to 250 ml with distilled water.

B.7.9 **1M Tris-HCl Buffer pH 7.8**

Trizma base	12.1 g
Distilled water	50 ml

The pH was adjusted to 7.8 with concentrated hydrochloric acid, and the final volume was made up to 100 ml with distilled water.

C. Methods

C.1 Levels of the enzymes of ketone body utilisation in the tissues of NMR I mice

Levels of 3-hydroxybutyrate dehydrogenase, 3-oxo acid-CoA transferase and acetoacetyl-CoA thiolase, the three enzymes required for the utilisation of ketone bodies were measured in the tissues of NMR I mice.

C.1.1 Animals

Male and female NMR I mice were used in this study and were divided up into four groups, each containing four animals. Groups A and B contained male mice, and groups C and D contained female mice. The animals in groups A and C were non tumour-bearing, those in groups B and D had been implanted with MAC 16 tumour as described in Section B.6.1.

C.1.2 Preparation of tissue extracts

On day 24 after implantation of tumour, all animals were killed by cervical dislocation, weighed and then the following tissues quickly removed: heart, liver, kidneys, brain, colon, and tumour, from those animals with tumour. All tissues were weighed, chopped finely and homogenised in 4 volumes of 0.01 M Tris-HCl buffer pH 7.4, containing 0.25 M sucrose and 1 mM 2-mercaptoethanol using a CAMLAB 563C homogeniser (speed 6) fitted with a teflon pestle. The homogenates cooled in ice-water were then exposed to ultrasonic vibration for 30 sec using an MSE sonic oscillator and a 100 watts of power. The sonically treated homogenates were centrifuged for 20 min at 30,000 x g in an MSE Hi-spin 21 ultracentrifuge. The supernatant fluid, which was considered to contain the total soluble portion of

the cell, was then used for the determination of enzyme activity.

C.1.3 **Protein determination**

The protein content of the supernatant samples was determined using the method of Lowry et al (1951) with bovine serum albumin as standard.

C.1.4 **Determination of enzyme activities**

All enzyme assays were carried out in a Beckman DU7 spectrophotometer at a temperature of 25° C. The assay methods used were those described by Tisdale and Brennan (1983).

C.1.4.1 3-Hydroxybutyrate dehydrogenase

The level of 3-hydroxybutyrate dehydrogenase activity was determined by measuring the increase in absorbance at 340 nm due to the formation of NADH. The reaction cuvettes contained 16 mM Tris-0.32 mM hydrazine hydrate buffer pH 8.5, 16 mM 3-hydroxybutyrate and 0.45 mM NAD in a total volume of 3 ml. The reaction was initiated by the addition of sample (50 or 100 µl) and the increase in absorbance at 340 nm recorded for 60 min. The rate of reaction was calculated from the linear portion of the reaction curve.

C.1.4.2 3-oxo acid-CoA transferase

The level of activity of 3-oxo acid-CoA transferase was determined in both the forward and the backward direction, ie by measuring the rate of acetoacetyl-CoA formation from succinyl-CoA and acetoacetate at 313 nm, and the rate of succinyl-CoA formation at 303 nm. The principle of the assays is based on the work of Stern et al (1956).

C.1.4.2a Determination of 3-oxo acid-CoA transferase activity in the forward direction

Cuvettes were made up containing 50 mM Tris-HCl buffer pH 8.5, 5 mM $MgCl_2$, 5 mM iodoacetamide (to inhibit acetoacetyl-CoA thiolase), 0.1 mM succinyl-CoA and sample (up to 200 µl for tumour tissue) in a final volume of about 2 ml. The reaction was initiated by the addition of 100 µMol acetoacetate and the rate of increase in absorbance at 313 nm measured for 2 min. The rate of reaction was calculated from the linear portion of the reaction curve. Under these conditions the millimolar extinction coefficient was 12 (Williamson et al, 1971).

C.1.4.2b Determination of 3-oxo acid-CoA transferase activity in the backward direction

Cuvettes were made up containing 50 mM Tris-HCl buffer pH 8.5, 10 mM $MgCl_2$, 5mM iodoacetamide and 0.1 mM acetoacetyl-CoA in a final volume of 2 ml. The change in absorbance at 303 nm was measured for 2 min (spontaneous hydrolysis of acetoacetyl-CoA) and then up to 50 µl sample was added and the change in absorbance measured for a further 3 min. This represents spontaneous hydrolysis plus acetoacetyl-CoA deacylase activity. 100 µMol sodium succinate was added to the cuvette and the change in absorbance measured for a further 3 min, representing spontaneous hydrolysis plus deacylase plus 3-oxo acid-CoA transferase activity. The millimolar extinction coefficient of acetoacetyl-CoA was 20.5 under these conditions (Williamson et al, 1971).

C.1.4.3 Acetoacetyl-CoA thiolase

The activity of acetoacetyl-CoA thiolase was determined by measuring the decrease in absorbance at 303 nm due to the cleavage of acetoacetyl-CoA (Stern et al, 1956). Cuvettes were made up containing 50 mM Tris-HCl buffer pH 8.5, 5 mM $MgCl_2$, 75 μM acetoacetyl-CoA, 100 μM CoA and 50 mM KCl in a final volume of 2 ml. The reaction was initiated by the addition of sample (5-50 μl) and the decrease in absorbance measured for 2 min.

C.2 Metabolite Studies

In these studies the levels of various metabolites were measured in the blood of tumour-bearing and non tumour-bearing male NMR I mice. The aim of this was to see what changes, if any, occurred in the levels of the metabolites with the development of the tumour. Changes in body weight, carcass weight, tumour weight and body composition were also measured with the aim of assessing the suitability of the MAC 16 tumour model for the study of cachexia. In each study mice from both groups were randomly selected and sacrificed at regular intervals over a 3-4 week period after implantation of MAC 16 tumour into tumour-bearing animals. All animals were weighed at the start of each study and on the day of termination. Carcass weights and tumour weights were recorded for those animals sacrificed after collection of blood samples and the carcasses stored at -20° C until ready to assay for body composition.

C.2.1 Collection of blood samples

Mice were first anaesthetised using a mixture of halothane,

oxygen and nitrous oxide (halothane 2.5%, O_2 0.5 cc/min, N_2O 0.7 cc/min). Approximately 1 ml of blood was collected after cardiac puncture into syringes containing 0.05 ml heparin solution (15 mg/ml). Blood samples were then transferred to microfuge tubes on ice and the levels of the following metabolites measured:

Glucose

3-Hydroxybutyrate

Acetoacetate

Lactate

Pyruvate

Glycerol

Carnitine

Free fatty acid

Insulin

Amino acid profiles.

C.2.2 **Determination of glucose**

Glucose levels were determined in samples of whole blood using the Sigma O-Toluidine reagent kit (No 635) which is based on the work of Hyvarinen and Nikkila (1962) and Feteris (1965). In the presence of heat and acid O-Toluidine reacts readily with glucose to form a blue-green complex, the intensity of which may be measured at 635 nm using a spectrophotometer. The intensity of the colour found is proportional to the glucose concentration. All measurements were carried out using a Beckman DU7 spectrophotometer.

C.2.3 Determination of 3-hydroxybutyrate and acetoacetate

C.2.3.1 Preparation of blood samples

Immediately after collection 100 µl whole blood was transferred to a microfuge tube on ice and deproteinised by the addition of 100 µl ice-cold 10% perchloric acid. After thorough mixing, the deproteinised sample was sedimented in a Beckman microfuge for 30 sec. The supernatant was carefully transferred to a clean microfuge tube and the pH adjusted to 7 with 20% w/v potassium hydroxide solution. The neutralised supernatant was allowed to stand in an ice-bath for approximately 30 min before being sedimented in a Beckman microfuge for 30 sec. The supernatant was carefully decanted and used for the determination of 3-hydroxybutyrate and acetoacetate.

C.2.3.2 3-Hydroxybutyrate

The level of 3-hydroxybutyrate was determined using the method of Williamson and Mellanby (1974) which depends on the oxidation of 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase and NAD. The increase in absorbance at 340 nm due to the formation of NADH is a measure of the reaction. Assay cuvettes were made up containing 16 mM Tris-0.32 mM hydrazine hydrate buffer pH 8.5, 0.45 mM NAD and sample (25-50 µl) in a total volume of 3 ml. The reaction was initiated by the addition of 10 µl 3-hydroxybutyrate dehydrogenase (150 mU) and the increase in absorbance measured for 60 min at 340 nm and a temperature of 25° C in a Beckman DU7 spectrophotometer.

C.2.3.3 Acetoacetate

The level of acetoacetate was determined using the method of Mellanby and Williamson (1974) in which the decrease in absorbance at

340 nm due to the oxidation of NADH is proportional to the amount of acetoacetate present. Assay cuvettes were made up containing 33 mM phosphate buffer pH 6.8, 0.2 mM NADH and sample (25-50 µl) in a total volume of 3 ml. The reaction was initiated by the addition of 10 µl 3-hydroxybutyrate dehydrogenase (150 mU/ml) and the decrease in absorbance measured for 20 min at 340 nm and a temperature of 25° C in a Beckman DU7 spectrophotometer.

C.2.4 **Lactate**

C.2.4.1 Preparation of blood samples

50 µl of whole blood was deproteinised by the addition of 100 µl 1 M perchloric acid. After thorough mixing the deproteinised sample was sedimented in a Beckman microfuge for 30 sec. The supernatant was used for the determination of lactate.

C.2.4.2 Determination of lactate

The level of lactate was determined using the method of Gutmann and Wahlefield (1974) which depends on the oxidation of lactate to pyruvate by NAD in the enzymatic reaction catalysed by lactate dehydrogenase. The increase in absorbance at 340 nm due to the formation of NADH is a measure of the reaction. Assay cuvettes were made up containing 0.43 M glycine - 0.34 M hydrazine hydrate buffer pH 9.0, 2.75 mM NAD and sample (25-50 µl) in a total volume of 3 ml. A blank was prepared containing 1 M perchloric acid in place of sample. The reaction was initiated by the addition of 20 µl lactate dehydrogenase (60 U) and the increase in absorbance measured for 30 min at 340 nm and a temperature of 37° C in a Beckman DU7 spectrophotometer.

C.2.5 Pyruvate

C.2.5.1 Preparation of blood samples

100 µl of whole blood was deproteinised by the addition of 300 µl ice-cold 0.6 M perchloric acid. After thorough mixing the deproteinised sample was left to stand for 10 min in an ice-bath and was then sedimented in a Beckman microfuge for 30 sec. The pH of the supernatant was adjusted to around 4 with 2 M KOH and then left to stand for 10 min to allow precipitation of potassium perchlorate. The sample was then recentrifuged in a Beckman microfuge for 30 sec and the supernatant removed from the potassium perchlorate pellet and used for the assay of pyruvate. Pyruvate is unstable so assays were carried out as soon as possible on the supernatant.

C.2.5.2 Determination of pyruvate

The level of pyruvate was determined using the method of Czok and Lamprecht (1974) in which pyruvate is converted enzymatically to lactate, with the oxidation of NADH. The oxidation of NADH is proportional to the amount of pyruvate converted and is measured at 365 nm. Assay cuvettes were made up containing 300 mM triethanolamine buffer pH 7.6, 3 mM EDTA, 0.1 mM NADH and sample (50 - 100 µl) in a total volume of 2 ml. The reaction rate was initiated by the addition of 20 µl lactate dehydrogenase (5.5 U) and the change in absorbance measured for 25 min at 365 nm and at a temperature of 25 °C in a Beckman DU7 spectrophotometer.

C.2.6 Glycerol

C.2.6.1 Preparation of blood samples

100 µl of whole blood was deproteinised by the addition of 100 µl ice-cold 0.5 M perchloric acid. After thorough mixing the deproteinised sample was sedimented in a Beckman microfuge for 30 sec. The supernatant was transferred to a clean microfuge tube on ice and the precipitate washed with a further 100 µl ice-cold 0.5 M perchloric acid, recentrifuged and the washing combined with the first supernatant. The pH of the supernatant plus washings was adjusted to 9 - 9.5 with 10 M KOH and then left to stand on ice for 10 min before being recentrifuged in a Beckman microfuge for 30 sec. The clear supernatant was then used for the assay of glycerol.

C.2.6.2 Determination of glycerol

The level of glycerol was determined using the method of Wieland (1974) in which glycerol is converted to glycerophosphate by ATP and glycerokinase. The oxidation of the glycerophosphate by the NAD-dependent glycerophosphate dehydrogenase serves as the indicator reaction. The formation of NADH as measured by the change in absorbance at 340 nm, is proportional to the amount of glycerol present. Assay cuvettes were made up containing 0.137 M glycine-0.67 M hydrazine hydrate buffer pH 9.8, 1.37 mM $MgCl_2$, 1.23 mM ATP, 0.49 mM NAD, 8 U glycerophosphate dehydrogenase and sample (50-100 µl) in a total volume of 2 ml. The reaction was initiated by the addition of 20 µl glycerokinase (17 U) and the increase in absorbance measured until constant at 340 nm and at a temperature of 25° C in a Beckman DU7 spectrophotometer.

C.2.7 Carnitine

C.2.7.1 Preparation of blood samples

100 µl of whole blood was deproteinised by the addition of 300 ul ice-cold 0.05 M perchloric acid. After thorough mixing, the deproteinised sample was sedimented in a Beckman microfuge for 30 sec, 10 µl 0.5 M phosphate buffer pH 7.5 was added to the supernatant and after mixing, the pH was adjusted to 6.5 - 7 with 1 M KOH. After standing on ice for 5 min the neutralised supernatant was centrifuged for 30 sec in a Beckman microfuge and the clear supernatant used for the assay of carnitine.

C.2.7.2 Determination of carnitine

Carnitine levels were determined using the DTNB [5,5'-dithiobis-(2-nitrobenzoate)] method of Pearson et al (1974) in which carnitine is acetylated to form a stoichiometric amount of coenzyme A in an assay system containing acetyl-CoA in excess and carnitine acyltransferase. The coenzyme A is then allowed to react further in a coupled irreversible reaction with DTNB to form a yellow anion, 5-thio-2-nitrobenzoate, which absorbs strongly at 412 nm and gives a quantitative measure of the amount of carnitine. Assay cuvettes were made up containing 100 uM Tris-HCl buffer pH 7.5, 1.25 mM EDTA, 0.15 mM acetyl-CoA, 0.125 mM DTNB and 100 µl sample in a total volume of 2 ml. The reaction was initiated by the addition of 10 µl carnitine acyltransferase (1200 mU) and the increase in absorbance at 412 nm and at a temperature of 25 °C measured for 6 min in a Beckman DU7 spectrophotometer.

C.2.8 **Free fatty acid levels**

C.2.8.1 Preparation of blood samples

Free fatty acid levels were determined in plasma obtained by centrifuging whole blood in a Beckman microfuge for 30 sec.

C.2.8.2 Determination of free fatty acid levels in plasma

A Wako NEKA C kit, which is an enzymatic colourimetric test for the *in vitro* assay of free fatty acids in serum and plasma, was used. Acyl-CoA synthetase in the presence of ATP and free fatty acids catalyses the acylation of CoA to acyl-CoA, which is oxidised by acyl-CoA oxidase to give 2,3-trans-enoyl-CoA with the formation of hydrogen peroxide. In the presence of peroxidase, 4-aminoantipyrine and 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) form a red quinone dye by oxidative condensation with hydrogen peroxide, the absorbance of which can be measured at 550 nm. The concentration of the dye is proportional to the concentration of free fatty acids. 20 μl of plasma was used in each assay.

C.2.9 **Insulin levels**

Plasma samples were prepared as described in section C.2.8.1 and then insulin levels were measured by Mr D Lambert in Biological Sciences using radioimmunoassay.

C.2.10 **Amino acid profiles**

Plasma samples were prepared as described in section C.2.8.1, and then pooled for each group of animals and sent to the Macro-

molecular Analysis Service at Birmingham University, where amino acid profiles were obtained for each sample using a Locarte Automatic Amino Acid Analyser.

C.2.11 **Determination of body composition**

The gastrocnemius muscle from the left hind leg of the carcass was carefully dissected out and weighed. Each carcass plus gastrocnemius muscle was placed in an oven at 80°C until constant weight was reached. The carcass plus muscle was reweighed separately to obtain the dry weight. The water content for the muscle and total carcass was calculated from the wet and dry weights. Total fat content of the carcass was determined using the method of Lundholm et al (1975). Each carcass was broken up into small pieces and extracted firstly with 2 x 25 ml volumes of acetone:ethanol (1:1 v/v) followed by 2 x 25 ml volumes of chloroform:methanol (1:1 v/v) and finally 2 x 25 ml volumes of diethyl ether. The extracts were combined and transferred to a pre-weighed round-bottomed flask and the solvents evaporated off using a Büchi rotary evaporator to leave a fatty residue. The flask was reweighed and the amount of fat extracted from the carcass calculated.

C.3 Diet studies

C.3.1 The effect of diet on tumour growth, body weight, body composition and metabolite levels in female NMR I mice

C.3.1.1 Diet treatments

In this study, five different diet treatments were used:

1. Normal pelleted diet, ie. rat and mouse breeding diet plus water.
2. 52.5% fat diet with 52.5% of the energy provided from fat, plus water.
3. 68% fat diet with 68% of the energy provided from fat, plus water.
4. 52.5% fat diet plus water plus 12 mg/mouse 3-hydroxybutyrate arginine salt, given orally in 0.3 ml water with a dosing syringe on a daily basis.
5. 68% fat diet, plus water, plus 3-hydroxybutyrate arginine salt given as above.

The fat diets were made up according to the recipes shown in Table C.3.1, which were formulated by Pilsburys Ltd.

C.3.1.2 Animals

Tumour-bearing and non tumour-bearing female NMR I mice were used in this study. Tumour-bearing animals were implanted with MAC 16 tumour in the way described previously in section B.6.1. On arrival mice were weighed and split up into 7 groups. Groups A-E contained tumour-bearing mice and groups F and G contained non tumour-bearing mice. All mice were fed on rat and mouse breeding diet and water ad-libitum for the [illegible] days prior to starting the diet

Table C.3.1 FORMULAE FOR MOUSE DIETS

Diets are isocaloric, isonitrogenous and with an increasing proportion of the energy from fat.

(NFE term is used as an indication of carbohydrate content and is calculated by difference at constant moisture).

ANALYSIS % ENERGY X FAT (CALC.)	11.48	52.5	68.0	80.0	
NFE	50.34	32.26	25.4	22.8	
RAW MATERIALS INCLUSION RATE					
Soya (dehulled)	.	275.74	349.2	361.2	
Limestone	.	5.46	7.45	9.1	
Bentonite (inert filler)	.	54.94	75.20	66.0	
Salt	.	4.40	5.83	5.8	
Dicalcium phosphate	.	27.94	38.97	40.3	
Methionine	.	0.18	1.28	1.9	
Sodium caesinate	.	11.03	26.47	.	
Rat and mouse breeding diet	1000.	286.11	34.07	.	
Rodent 006 premix	.	13.13	17.76	18.0	
Tri-glyceride emulsion	.	321.07	443.77	497.7	
TOTAL OF RAW MATERIALS	1000.	1000.	1000.	1000.	grams

(Inclusion rates of raw materials are in grams/kilo: Energy 1244 Kcal/lb, Protein 200 g/kg in all diets 11.48 MJ/kg)

treatments. This was to allow the animals to settle down and adjust to their new surroundings.

On day 8 after implantation of tumour the different diet treatments were started. The groups of mice and their corresponding diet treatments are shown in Table C.3.2. Throughout the study all animals were weighed and food consumption monitored on each working day. The diet treatments were continued until day 28 after implantation of tumour when the study was terminated. All animals were weighed and then blood collected under anaesthesia as described previously in section C.2.1. The blood samples were pooled for each group of animals and the following metabolites measured:

Glucose

3-Hydroxybutyrate

Acetoacetate

Lactate

Free fatty acid

The methods of determination used for these metabolites were those described in sections C.2.2 - C.2.9.

Carcass weights and tumour weights were also recorded, and the tumours prepared as described in section C.1.2., and the level of 3-oxo acid-CoA transferase measured as described in section C.1.4.2. Total carcass fat and water content were determined in the carcasses using the methods described in section C.2.11.

Table C.3.2

GROUP	ANIMALS	DIET TREATMENT
A	Tumour-bearing female NMR I mice	Normal pelleted diet and water
B	" "	52.5% fat diet and water
C	" "	52.5% fat diet and water and 12 mg per mouse 3-hydroxybutyrate, arginine salt daily
D	" "	68% fat diet and water
E	" "	68% fat diet and water and 12 mg per mouse 3-hydroxybutyrate, arginine salt daily
F	Non tumour-bearing female NMR I mice	Normal pelleted diet and water
G	" "	68% fat diet and water and 12 mg per mouse 3-hydroxybutyrate, arginine salt daily

C.3.2 The effect of diet on tumour growth, body weight, body composition and metabolite levels in male NMR I mice

C.3.2.1 Diet treatments

In this study, six different diet treatments were used:

1. Normal pelleted diet, ie; rat and mouse breeding diet plus water
2. Normal pelleted diet plus water plus 20% sucrose
3. 68% fat diet with 68% of the energy provided from fat, plus water
4. 68% fat diet plus 3-hydroxybutyrate, arginine salt, 3 mg/ml in the drinking water
5. 80% fat diet with 80% of the energy provided from fat, plus water
6. 80% fat diet, plus 3-hydroxybutyrate, arginine salt, 3 mg/ml in the drinking water.

The fat diets were made up according to the recipes shown in Table C.3.1.

C.3.2.2 Animals

Tumour-bearing and non tumour-bearing male NMR I mice were used in this study. Tumour-bearing animals were implanted with MAC 16 tumour as previously described in section B.6.1. On arrival mice were weighed and divided up into 12 groups. Groups A-F contained tumour-bearing mice and groups G-L contained non tumour-bearing mice. All mice were fed on rat and mouse breeding diet and water ad-libitum for the days prior to initiating the diet treatments.

The dietary study was initiated 8 days after tumour

implantation. The groups of mice and their corresponding diet treatments are shown in Table C.3.3. Throughout the study all animals were weighed, 3-hydroxybutyrate, sucrose and food consumption were monitored. The diet treatments were continued until day 24 after implantation of tumour when the study was terminated. All animals were weighed and then blood collected under anaesthesia as described previously in section C.2.1. Levels of the following metabolites were measured in the blood samples using the methods described in sections C.2.2 - C.2.9:

Glucose

3-Hydroxybutyrate

Acetoacetate

Lactate

Free fatty acid

Insulin

Pooled plasma samples from each group were sent for amino acid analysis to the Macromolecular Analysis Service at Birmingham University. Carcass weights and tumour weights were recorded. Total carcass fat and water content was determined as described previously in section C.2.11. Tumours from each group were kept for histological examination.

C.3.2.3 Preparation of tumours for histological examination

After removal from the animals, tumours were weighed and then fixed in 10% formal saline. The tumours were prepared for histological examination by Miss R Holt. After fixation, the tumours were processed through graded alcohols and finally xylene and then embedded in Paramat wax. Sections, 4 μm thick, were cut and then stained with haematoxylin and eosin.

Table C.3.3

GROUP	ANIMALS	DIET TREATMENT
A	Tumour-bearing male NMR I mice	Normal pelleted diet and water
B	"	Normal pelleted diet and water and 20% sucrose
C	"	68% fat diet and water
D	"	68% fat diet and 3-hydroxybutyrate arginine salt, 3 mg/ml in drinking water
E	"	80% fat diet and water
F	"	80% fat diet and 3-hydroxybutyrate arginine salt, 3 mg/ml in drinking water
G	Non tumour-bearing male NMR I Mice	Normal pelleted diet and water
H	"	Normal pelleted diet and water and 20% sucrose
I	"	68% fat diet and water
J	"	68% fat diet and 3-hydroxybutyrate arginine salt, 3 mg/ml in drinking water
K	"	80% fat diet and water
L	"	80% fat diet and 3-hydroxybutyrate arginine salt, 3 mg/ml in drinking water

C.4 Statistical analysis of results

The results from all of the studies were analysed statistically where possible using the standard t-test.

D. Results

D.1 Enzyme studies

The levels of activity of the three enzymes required for the utilisation of ketone bodies described in section A.3, were measured in different tissues from male and female mice both with or without MAC 16 tumour. The levels of activity measured are shown in Tables D.1.1 and D.1.2, and displayed in histogram form in figures D.1.1 - D.1.8. The structure of the MAC 16 tumour was described briefly in section A.4 and its histological structure is shown in figure A.4.1. It is a moderately well-differentiated adenocarcinoma of the colon as shown by the presence of acini, which are a characteristic of colonic epithelium. Levels of activity of the three enzymes were measured in the tumour tissue and compared with normal colon, which was considered to be an appropriate control, taken from the same animals. The tissue distribution of the enzymes is similar to that previously reported (Tisdale and Brennan, 1983). No significant differences were found in the activity of these enzymes from the tissues of tumour-bearing and non tumour-bearing mice with the exception of colon.

D.1.1 Activity of 3-hydroxybutyrate dehydrogenase

The activity of this enzyme was highest in the kidney, and high levels were also found in heart and tumour. This latter observation suggests that the level of activity of 3-hydroxybutyrate dehydrogenase in the MAC 16 tumour is sufficient to allow for the utilisation of 3-hydroxybutyrate by metabolic oxidation. Levels of activity of this enzyme were similar in tissues from both male and female mice.

D.1.2 Activity of 3-oxo acid-CoA transferase

The levels of activity of 3-oxo acid-CoA transferase were highest in heart and lowest in the liver as previously reported (Fenselau and Wallis, 1974; Tisdale and Brennan, 1983). The level of activity of the enzyme measured in heart from female mice was significantly higher than the level found in heart from male mice ($p<0.02$). When tumour tissue is compared with normal colon, which is considered to be an appropriate control since the MAC 16 is a moderately well-differentiated adenocarcinoma of colonic origin, levels of activity in the MAC 16 tumour were significantly lower than those found in normal colon ($p<0.02$). The lowest levels of enzyme activity were found in liver, an organ not regarded as utilising ketone bodies as metabolic substrates. This result suggests that the MAC 16 tumour may have a limited capacity to utilise acetoacetate as a metabolic substrate.

As in previous studies with rat and mouse tissues (Fenselau and Wallis 1974; Tisdale and Brennan, 1983), the level of activity of the enzyme measured in the direction of acetoacetate synthesis (backward reaction, section C.1.4.2b) was about 9x higher than in the direction of acetoacetyl-CoA formation (forward reaction, section C.1.4.2a).

D.1.3 Activity of acetoacetyl-CoA thiolase

High levels of activity of acetoacetyl-CoA thiolase were found in kidney, heart and liver. Lowest levels were found in tumour, and this activity is significantly lower than that measured in normal colon ($p<0.003$). Levels of activity of the enzyme measured in heart

Table D.1.1 LEVELS OF ACTIVITY OF ENZYMES OF KETONE BODY UTILISATION IN THE TISSUES OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

TISSUE	3-HYDROXYBUTYRATE DEHYDROGENASE		3-OXO ACID CoA TRANSFERASE FORWARD		3-OXO ACID CoA TRANSFERASE BACKWARD		ACETOACETYL-CoA THIOLASE	
	µmole/min/ mg Protein	Relative % Activity Heart	µmole/min/ mg Protein	Relative % Activity Heart	µmole/min/ mg Protein	Relative % Activity Heart	µmole/min/ mg Protein	Relative % Activity Heart
A								
Heart	5.4±2.2	100	9.5±1.8[d]	100	77.4±14.0	100	42.8±6.3[d]	100
Liver	1.6±0.3	43.9	0.14±0.1	1.5	3.1±1.4	4.0	42.4±6.3[d]	99.1
Kidney	5.3±1.7	98.5	10.6±1.8	111.8	92.7±12.5	119.8	100±43.2	233.6
Brain	3.8±71.3	71.3	3.1±0.5	32.8	38.2±19.3	49.3	36.2±12.8	84.6
Colon	2.8±0.2	74.5	4.7±0.5	49.5	44.9±1.1	57.9	44±6.1	103
Tumour	5.1±1.23	93.5	1.6±0.5[a,c]	17.6	20.4±1.8[a]	26.3	16.4±2.2[a,b]	38.2

Table D.1.1 continued

B

Heart	1.1±0.3	100	11.3±3.4	100	69.4±28.3	100	45.7±1.3	100
Liver	1.1±0.3	100	0	0	4.9±1.5	7.13	60±13.5	131.3
Kidney	3.3±0.9	296	3.5±2.6	75	39.5±17.4	56.9	61.5±6.1	134.5
Brain	5.1±1.7	457	1.5±1.0	12.8	22.7±5.8	32.7	15.2±4.6	33.2
Colon	3.6±1.3	319	4.4±0.7	39.1	35.6±8.8	51.3	42.2±4.5	92.4

a) $p<0.003$ from colon in tumour-bearing mice

b) $p<0.003$ from colon in non tumour-bearing mice

c) $p<0.02$ from colon in tumour-bearing mice

d) $p<0.02$ from corresponding tissue in male tumour-bearing mice

Table D.1.2 LEVELS OF ACTIVITY OF ENZYMES OF KETONE BODY UTILISATION IN THE TISSUES OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

TISSUE	3-HYDROXYBUTYRATE DEHYDROGENASE		3-OXO ACID CoA TRANSFERASE FORWARD		3-OXO ACID CoA TRANSFERASE BACKWARD		ACETOACETYL-CoA THIOLASE	
	μmole/min/ mg Protein	Relative % Activity Heart	μmole/min/ mg Protein	Relative % Activity Heart	μmole/min/ mg Protein	Relative % Activity Heart	μmole/min/ mg Protein	Relative % Activity Heart
A								
Heart	2.9±1.0	100	24.7±4.2	100	360±100	100	105.1±18.9	100
Liver	1.8±0.2	63.4	0.3±0.2	1.2	3.3±0.6	0.92	87.6±8.2	83.4
Kidney	4.2±1.3	144.7	6.1±2.1	24.5	192±12.3	53.3	77.6±10.6	73.9
Brain	2.3±0.3	80.8	3.2±0.7	13.0	47.6±19.4	13.2	18.3±4.1	17.4
Colon	4.1±1.0	141.4	1.9±0.8	7.7	19.6±19.4	5.4	30.7±4.3[a,c]	29.3
Tumour	4.6±0.8	156	1.7±0.3[b]	7.1	26.7±8.9[b]	7.4	12.8±3.0	12.2

Table D.1.2 continued

B

Heart	1.3±0.2	100	14.2±2.2	100	283±49.9	100	33.8±12.3	100
Liver	2.9±0.6	229	0.3±0.2	2.3	7.6±2.2	2.7	66.8±19.9	197.7
Kidney	2.1±0.6	181.5	13.9±3.6	97.9	142.2±47.8	50.3	30.4±11.5	89.9
Brain	6.6±2.3	519	5.0±1.8	32.9	96.2±24.7	34.0	22.0±6.6	65.2
Colon	1.0±0.2	80.1	3.9±1.6	27.9	41.3±19.6	14.6	16.8±4.9	49.7

a) $p<0.003$ from colon in non tumour-bearing mice

b) $p<0.02$ from colon in non tumour-bearing mice

c) $p<0.003$ from colon in tumour-bearing mice

Figure D.1.1 LEVELS OF ACTIVITY OF 3-HYDROXYBUTYRATE DEHYDROGENASE IN THE TISSUES OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)

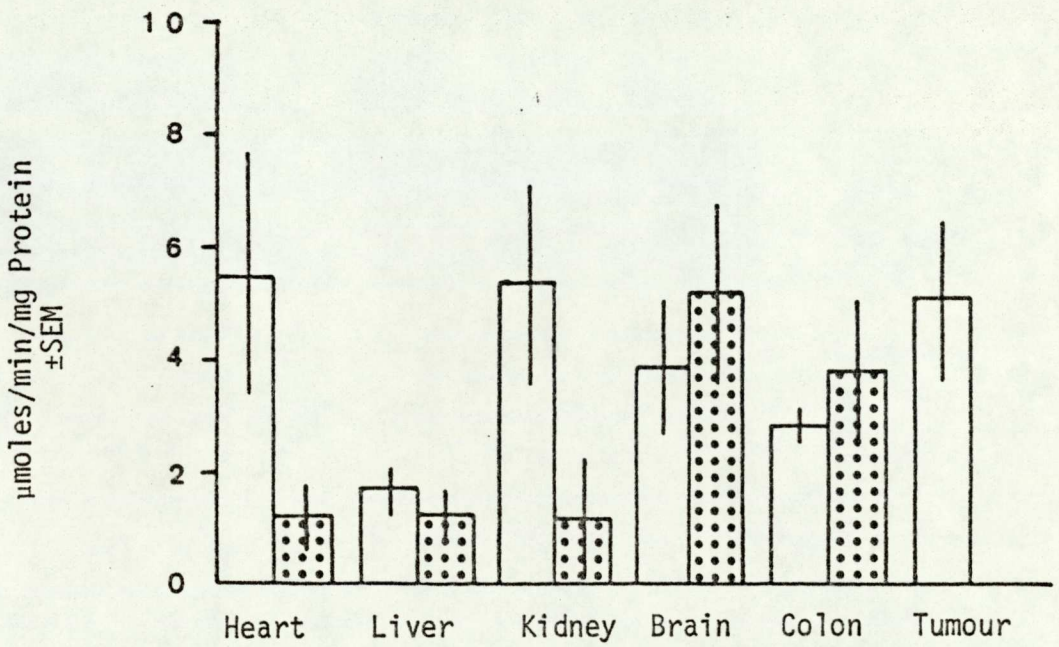
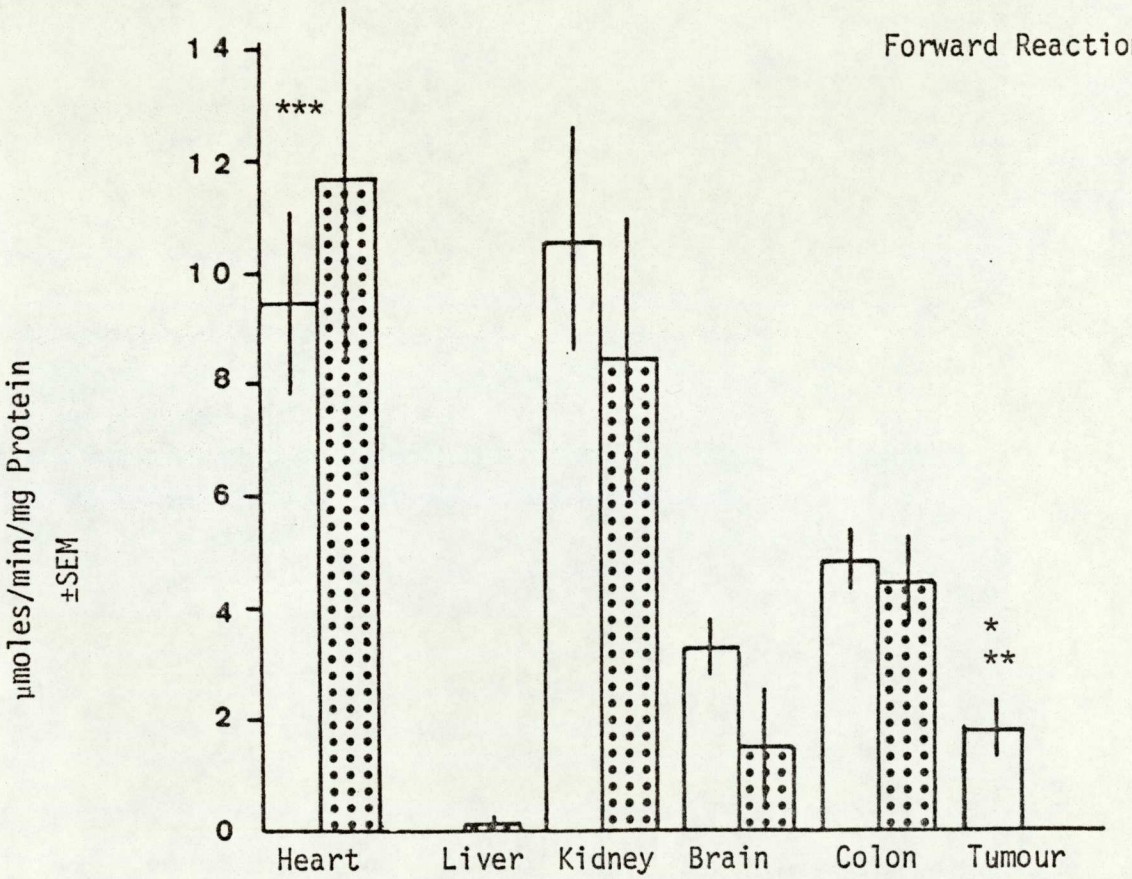


Figure D.1.2 LEVELS OF ACTIVITY OF 3-OXO ACID-CoA TRANSFERASE IN THE TISSUES OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)

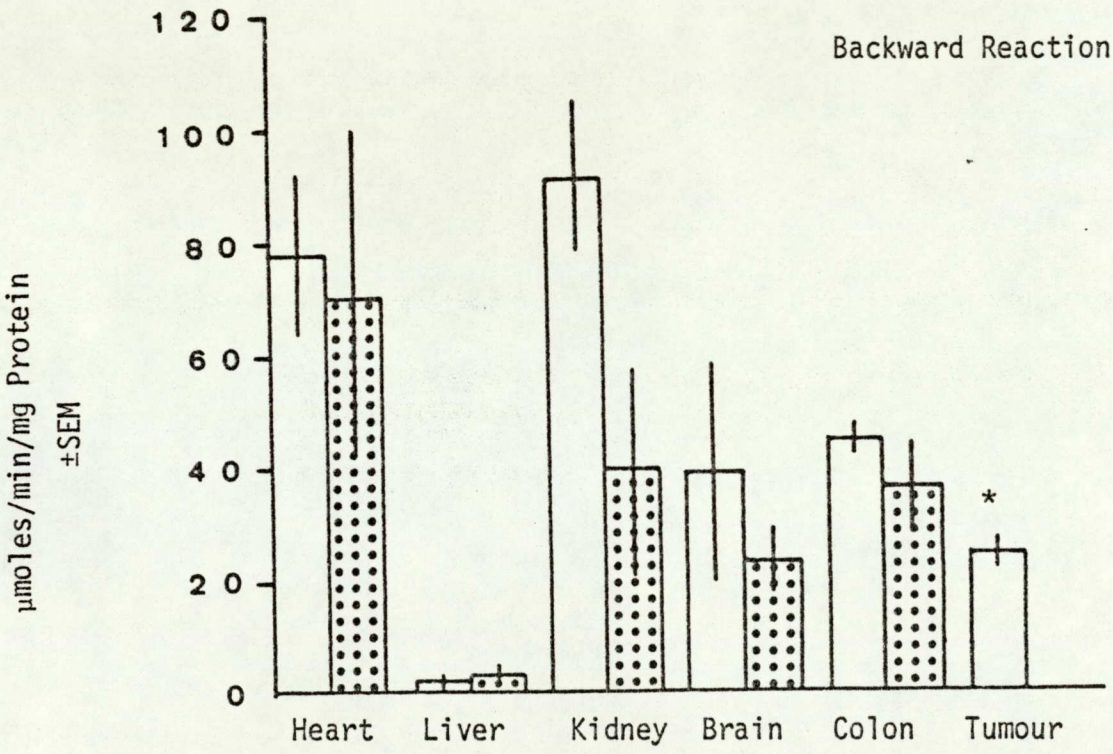


* p < 0.003 from colon in tumour-bearing mice

** p < 0.02 from colon in non tumour-bearing mice

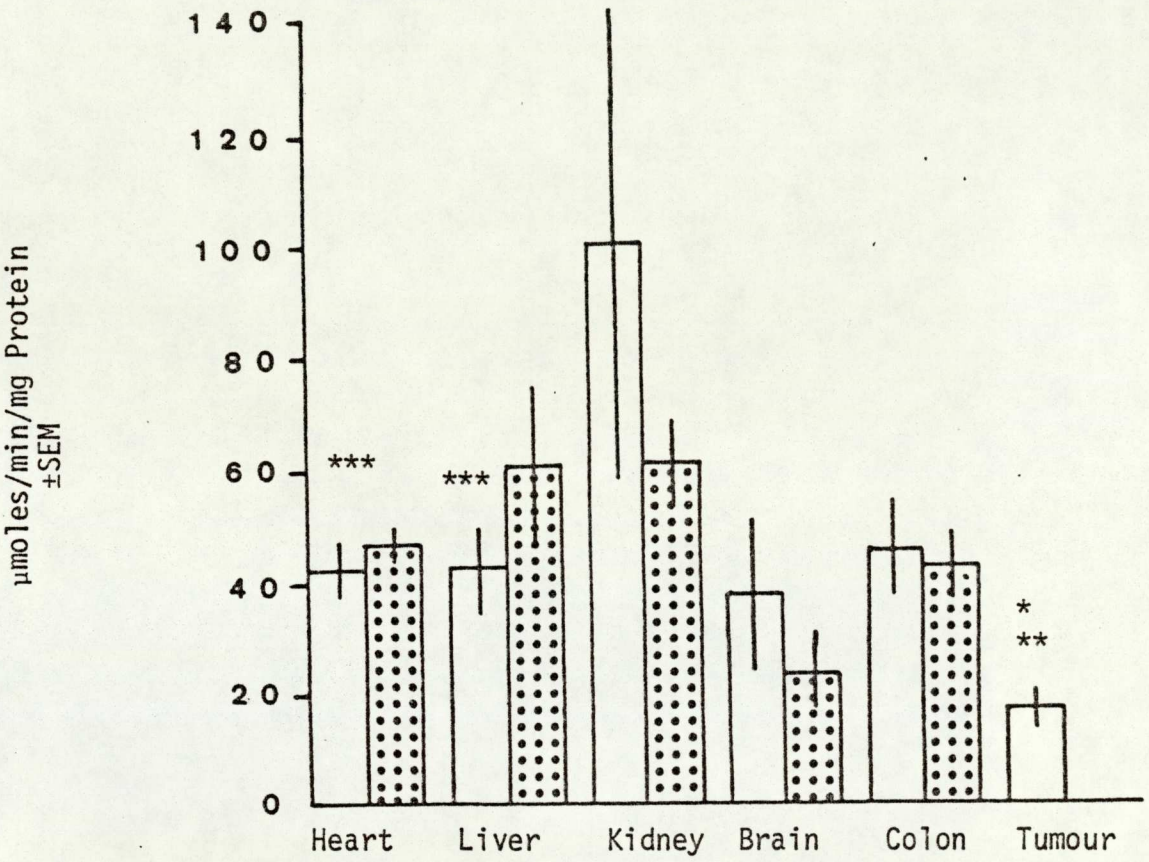
*** p < 0.02 from corresponding tissue in male tumour-bearing mice

Figure D.1.3 LEVELS OF ACTIVITY OF 3-OXO ACID-CoA TRANSFERASE IN THE TISSUES OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)



* $p < 0.003$ from colon in tumour-bearing mice

Figure D.1.4 LEVELS OF ACTIVITY OF ACETOACETYL-CoA THIOLASE IN THE TISSUES OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)



* $p < 0.003$ from colon in tumour-bearing mice

** $p < 0.003$ from colon in non tumour-bearing mice

*** $p < 0.02$ from corresponding tissue in male tumour-bearing mice

Figure D.1.5 LEVELS OF ACTIVITY OF 3-HYDROXYBUTYRATE DEHYDROGENASE IN THE TISSUES OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)

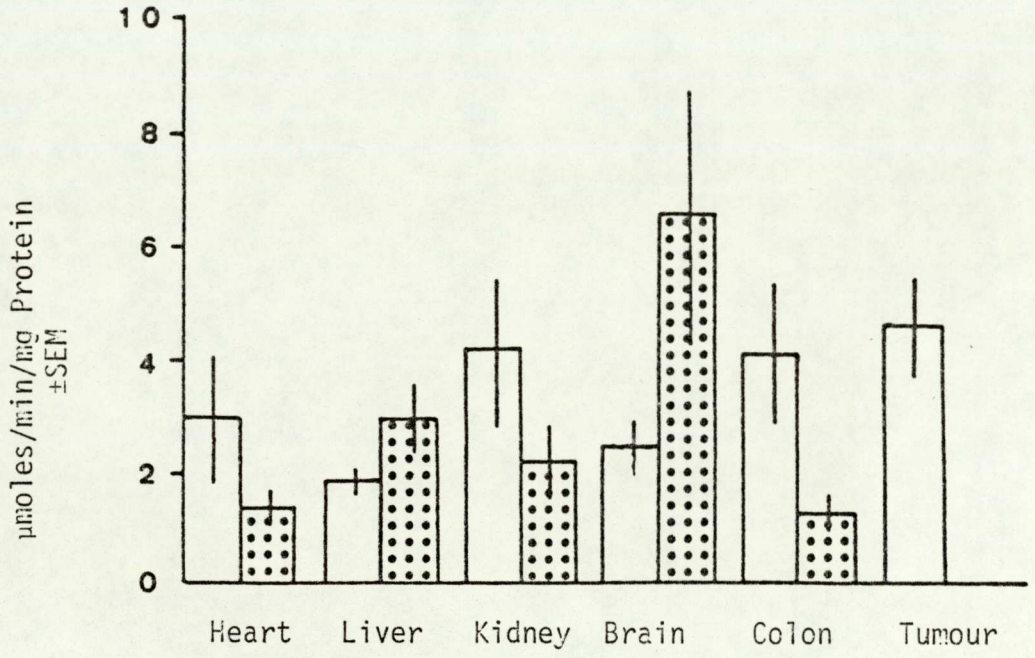
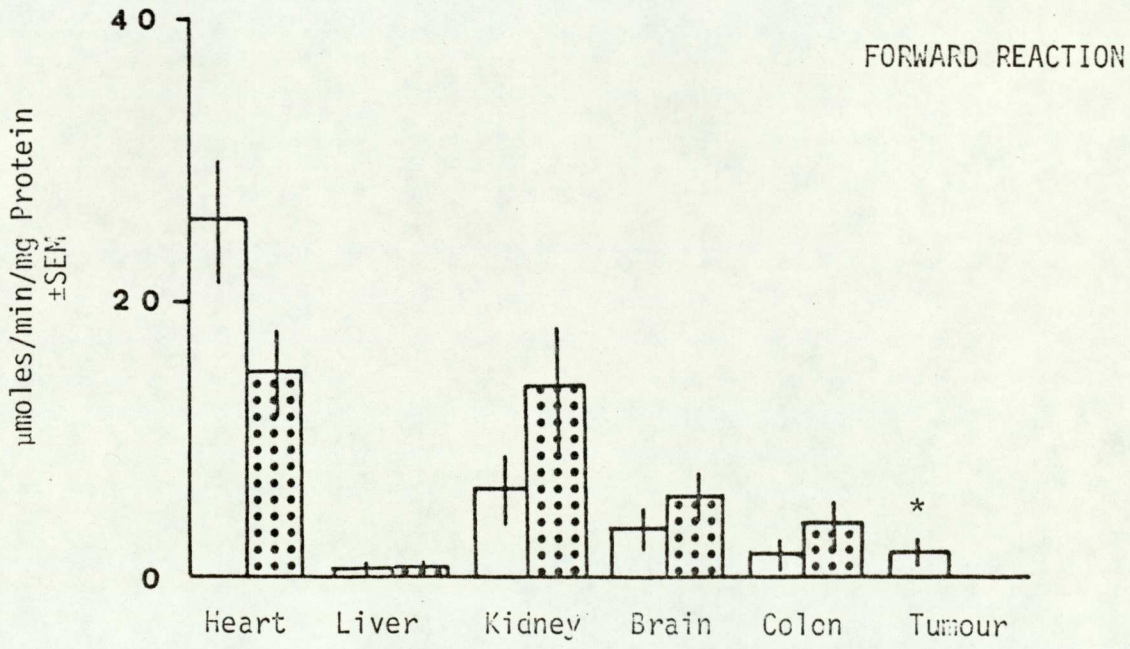
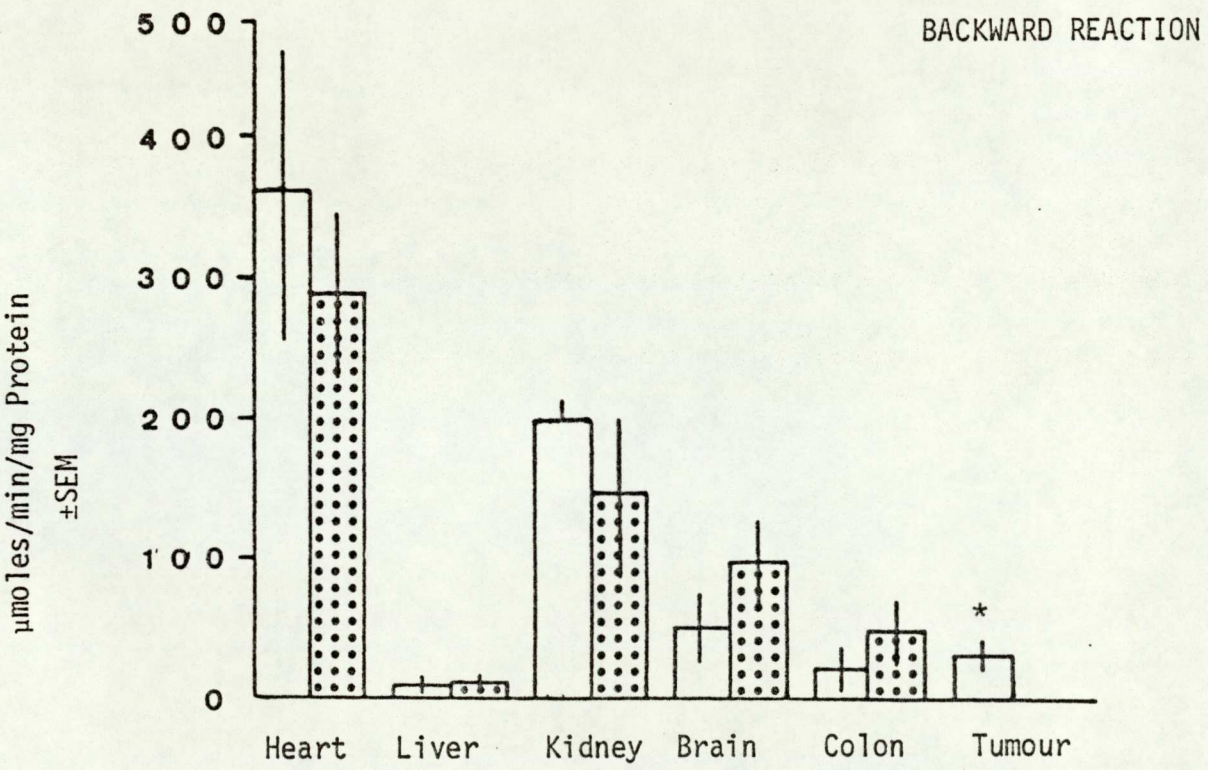


Figure D.1.6 LEVELS OF ACTIVITY OF 3-OXO ACID-CoA TRANSFERASE IN THE TISSUES OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)



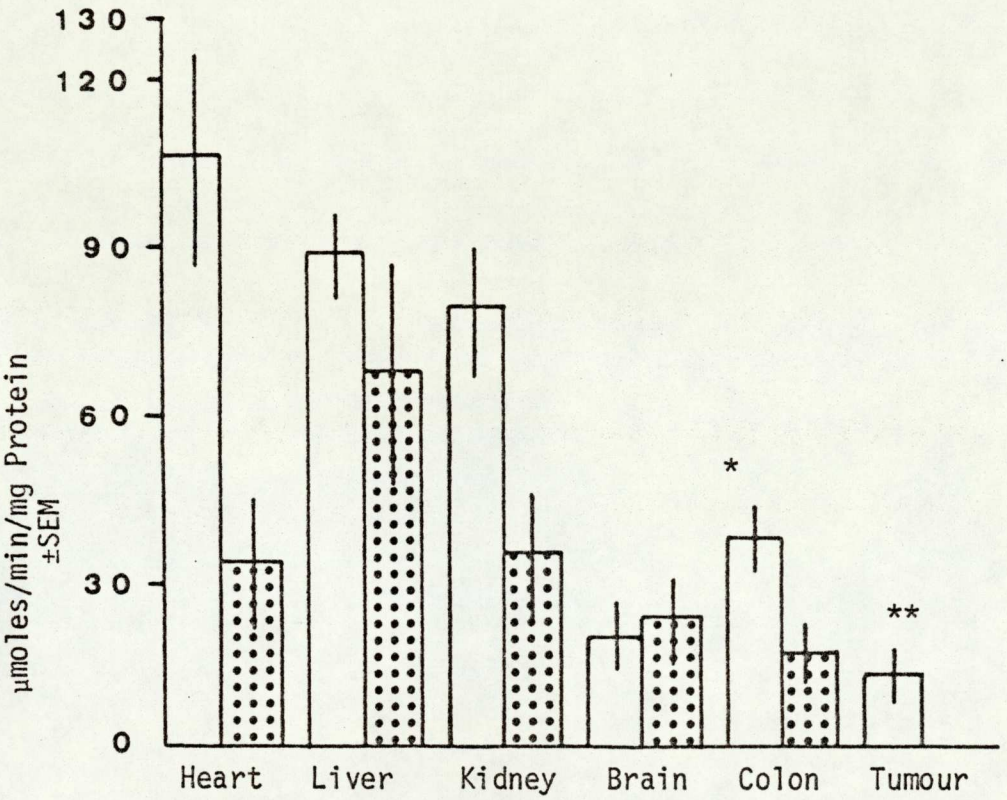
* $p < 0.02$ from colon in non tumour-bearing mice

Figure D.1.7 LEVELS OF ACTIVITY OF 3-OXO ACID-CoA TRANSFERASE IN THE TISSUES OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)



* $p < 0.02$ from colon in non tumour-bearing mice

Figure D.1.8 LEVELS OF ACTIVITY OF ACETOACETYL-CoA THIOLASE IN THE TISSUES OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (HATCHED BOXES)



* $p < 0.003$ from colon in non tumour-bearing mice

** $p < 0.003$ from colon in tumour-bearing mice

and liver from female mice were significantly lower ($p<0.02$) than those found in the corresponding tissues in male mice.

The high level of activity of acetoacetyl-CoA thiolase in liver probably reflects the role of the thiolase in processes other than ketone body utilisation ie; fatty acid oxidation and cholesterol syntheis (Williamson et al, 1971).

The results from this study show that the MAC 16 tumour displays a low level of activity of 3-oxo acid-CoA transferase (figure A.3.10) the key enzyme required for the utilisation of ketone bodies by the tissues as metabolic substrates, and therefore might be expected to metabolise acetoacetate and 3-hydroxybutyrate at a reduced rate.

D.2 Metabolite Studies

D.2.1 Total body weight

The total body weights for tumour-bearing and non tumour-bearing NMR I mice are shown in Table D.2.1, and the changes in total body weight over the duration of the study are shown in figure D.2.1. Figure D.2.2 shows the food consumption of both groups over the period of the study. There was little change in the total body weights of the non tumour-bearing mice over the period of the study, whilst those of the tumour-bearing mice fell and were significantly lower than those of the non tumour-bearing mice after day 17 ($p<0.05$). Food consumption was similar throughout the study for both groups of mice. Growth of the MAC 16 tumour is therefore accompanied by a loss in host body weight, and this may be as much as 20% of the total body weight 28 days after implantation of the tumour. Furthermore, this weight loss is not accompanied by a reduction in food

Table D.2.1 TOTAL BODY WEIGHTS OF NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	GRAMS ± SEM Non tumour-bearing mice	Tumour-bearing mice
0	31.5±0.85	32.2±0.98
7	32.4±0.55	32.1±0.34
14	32.5±0.57	30.9±0.73*
17	33.3±0.88	30.5±0.94* **
21	33.2±0.66	28.8±0.76* **
28	33.9±0.78	28.4±0.68* **

* $p<0.03$ from tumour-bearing group on day 0

** $p<0.05$ from non tumour-bearing groups of mice

Figure D.2.1 CHANGES IN TOTAL BODY WEIGHT OF NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (*) AND NON TUMOUR-BEARING MICE (●)

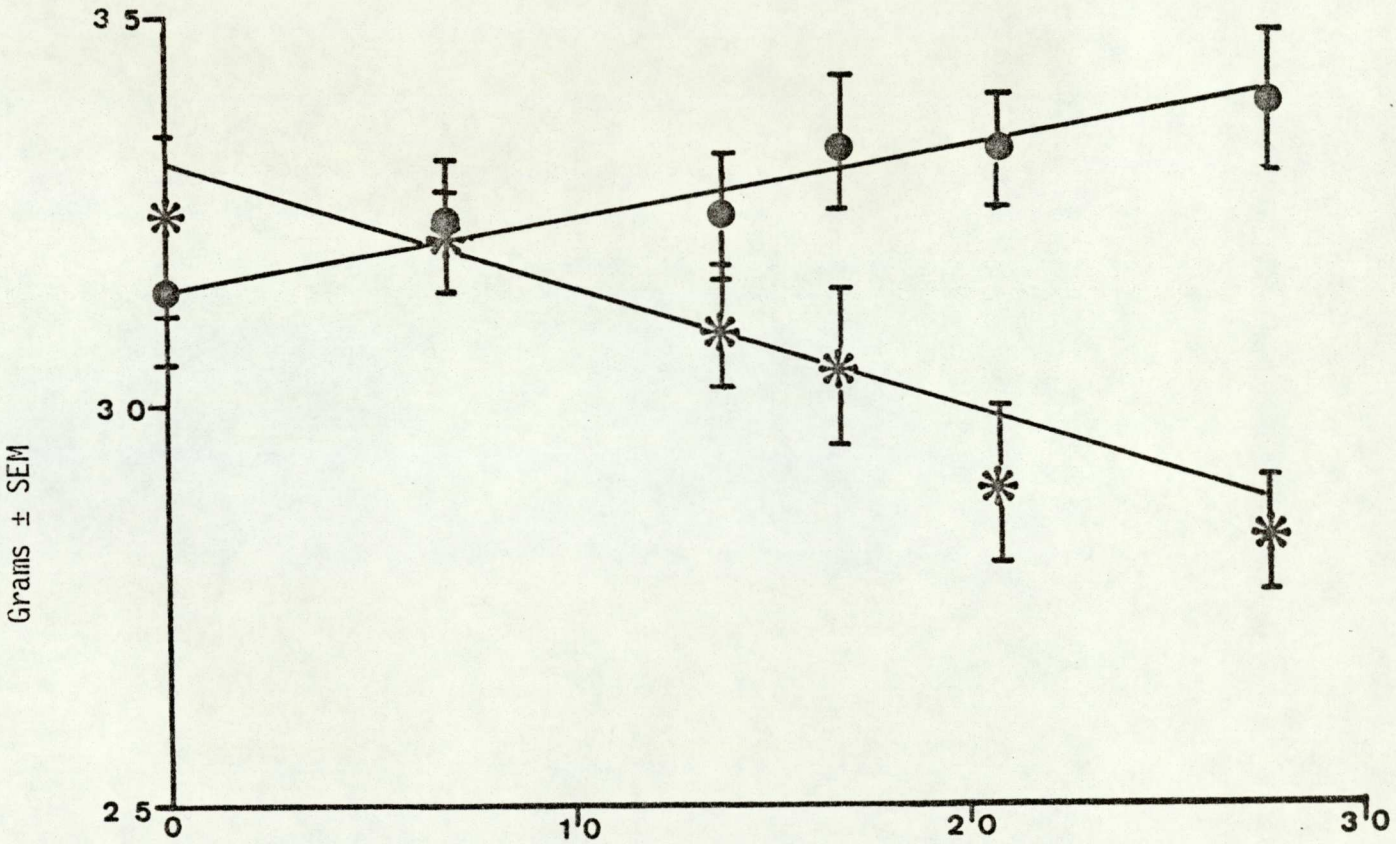
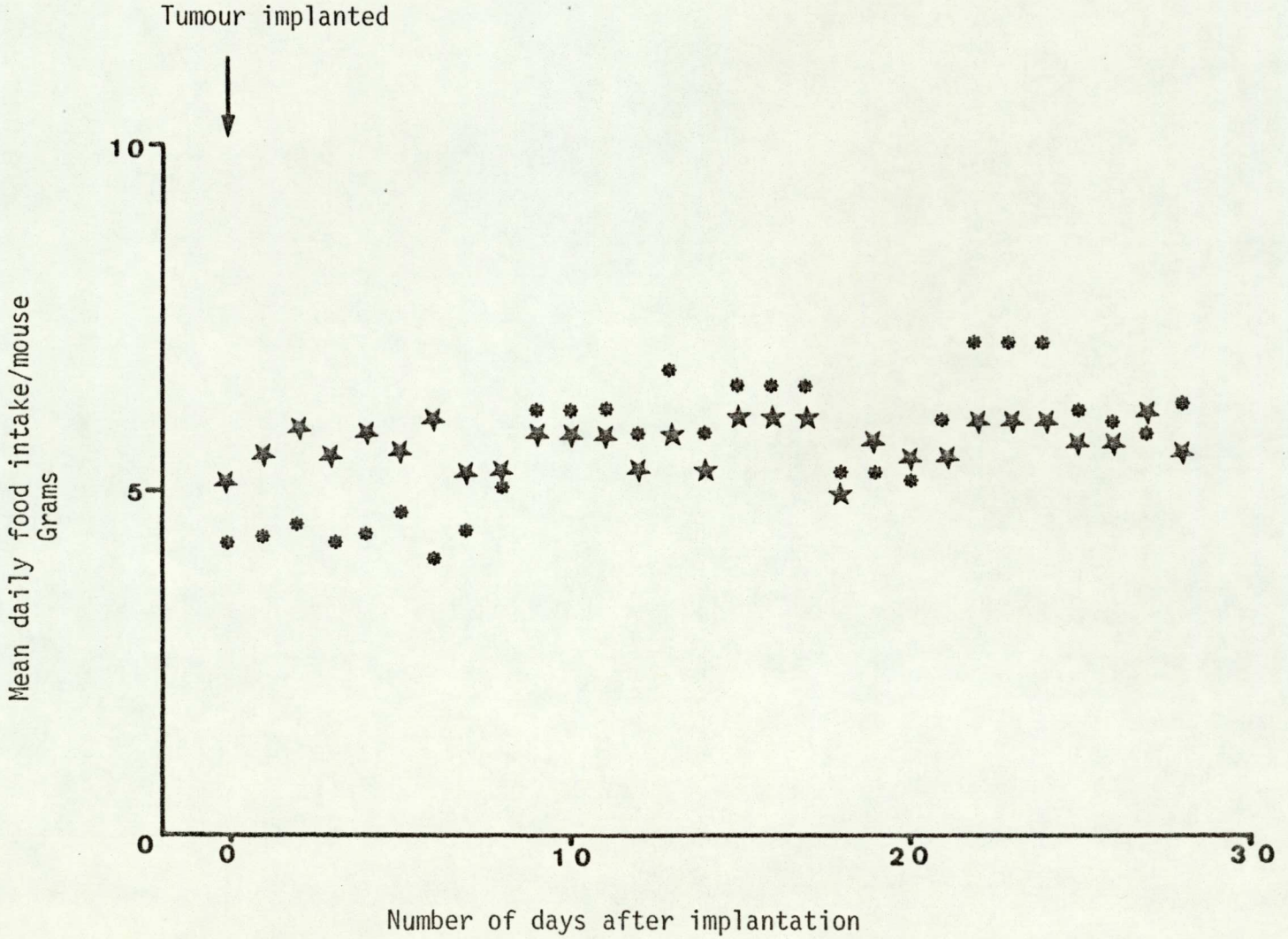


Figure D.2.2 FOOD CONSUMPTION OF NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (✱) AND NON TUMOUR-BEARING MICE (★) OVER THE DURATION OF THE METABOLITE STUDY



intake and therefore other factors, as yet unknown, must account for the weight reduction seen in the tumour-bearing mice.

D.2.2 Carcass weight and tumour weight

The carcass weights and tumour weights of the mice used in this study are shown in Table D.2.2, and changes in carcass weight and tumour weight over the duration of the study are shown in figure D.2.3. The relationship between carcass weight and tumour weight is shown in figure D.2.4. Carcass weight fell with increase in tumour weight, and this decrease in carcass weight is directly related to the size of the tumour. Furthermore, the tumour does not have to reach an extensive mass in order to produce weight loss in the host, since a significant weight reduction occurs in tumour-bearing animals 14 days after implantation of the tumour ($p<0.05$), at which time the tumour mass is only 0.3 gram, which is less than 1% of the host body weight. This weight reduction is clearly seen as a reduction in the size of the animal and disappearance of fat deposits (figure D.2.5).

D.2.3 Body composition

Body composition measured in terms of wet and dry carcass weight, total carcass fat and water content for tumour-bearing and non tumour-bearing mice is shown in Tables D.2.3 and D.2.4 respectively. Changes in these different components of body composition during the study are shown in figures D.2.6 - D.2.9. Wet and dry carcass weights of tumour-bearing mice were significantly lower than those of non tumour-bearing mice by day 21 after implantation of tumour ($p<0.05$). There was also a significant

Table D.2.2 CARCASS WEIGHTS AND TUMOUR WEIGHTS OF NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

Days after implantation with tumour	Mean Carcass Weight (grams)±SEM	Mean Tumour Weight (grams)±SEM
7	31.95±0.83	0.102±0.02
14	31.57±1.13	0.303±0.08
17	28.19±2.25	0.870±0.02
21	24.48±1.40* **	1.150±0.14
28	24.33±0.86* **	2.270±0.31

* $p<0.001$ from tumour-bearing group on day 7

** $p<0.01$ from non tumour-bearing groups of mice (Table D.2.4)

Figure D.2.3 CHANGES IN CARCASS WEIGHT (●) AND TUMOUR WEIGHT (✱) OF NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

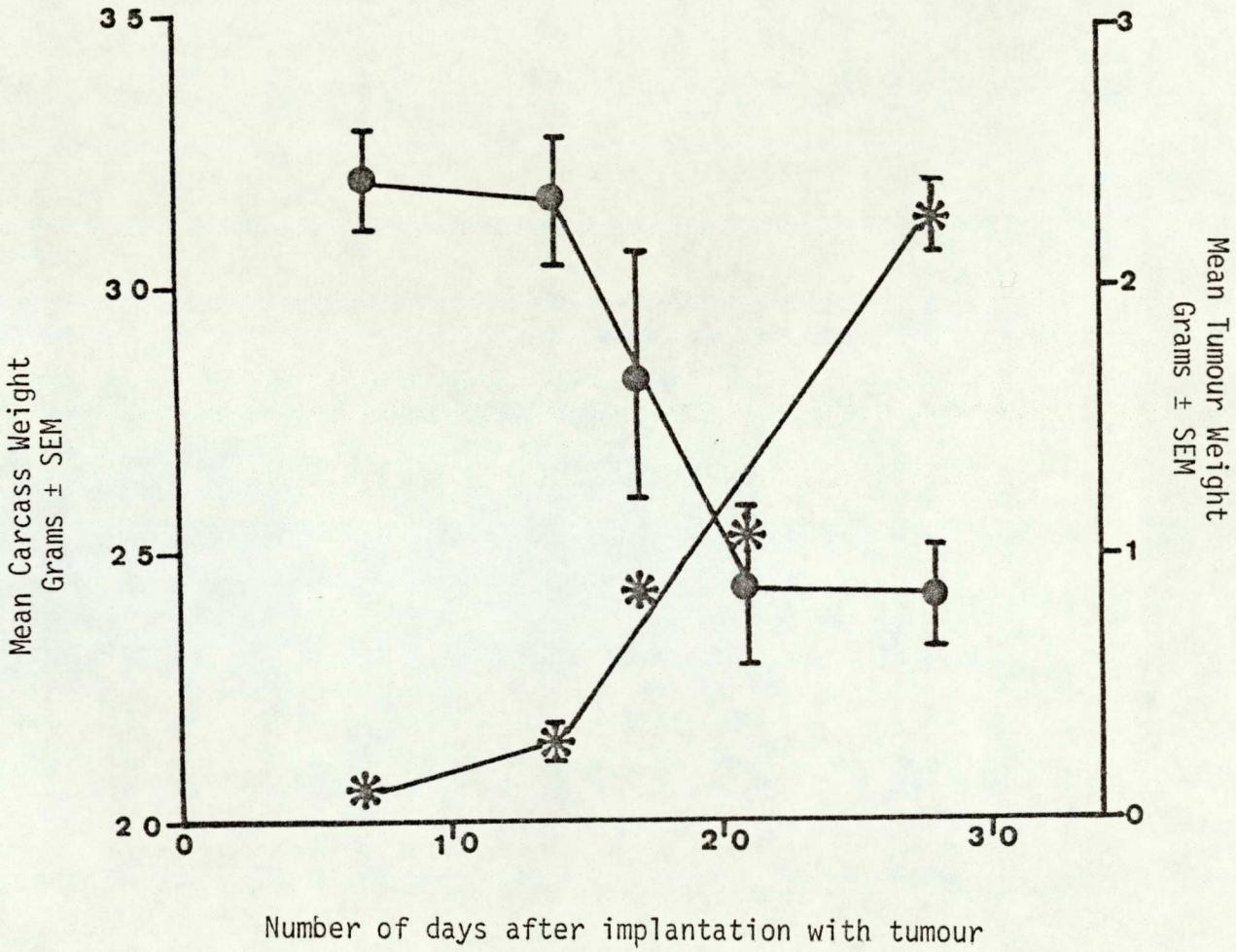
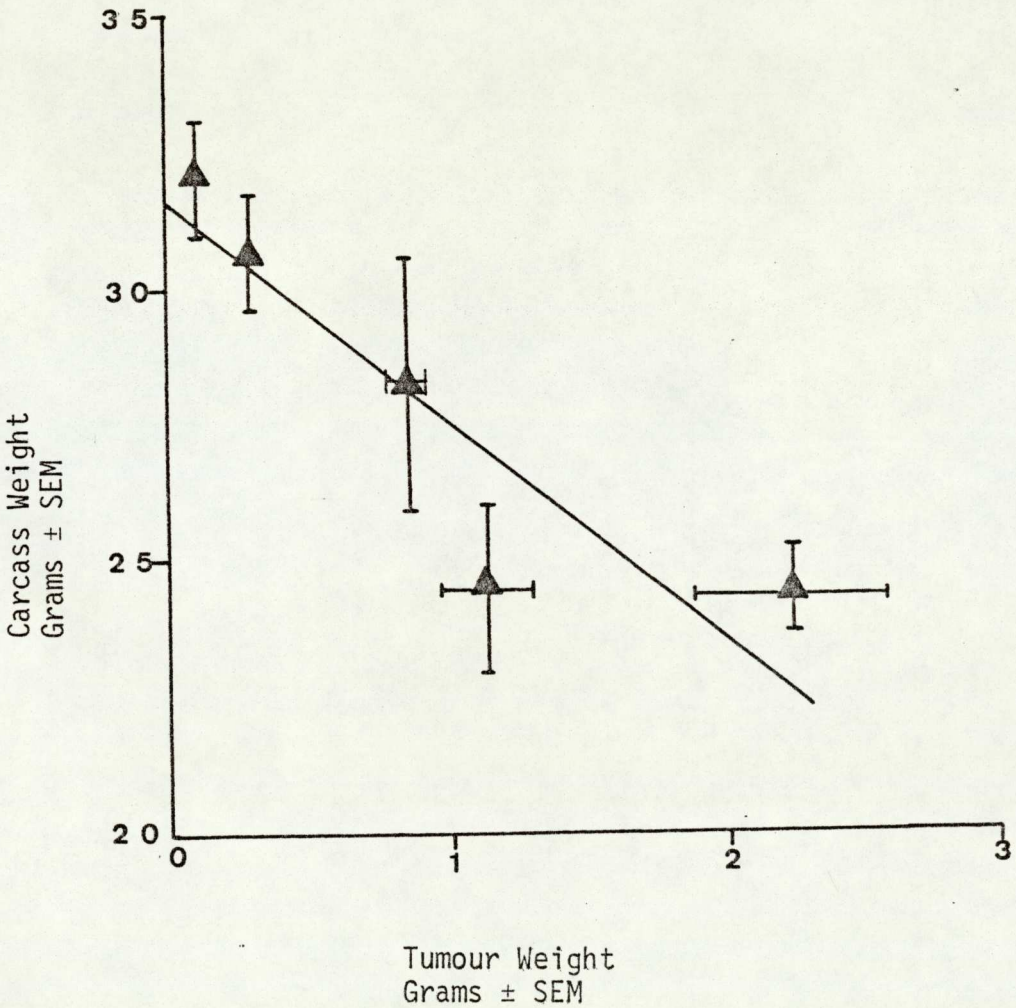


Figure D.2.4 THE RELATIONSHIP BETWEEN CARCASS WEIGHT AND TUMOUR WEIGHT IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA



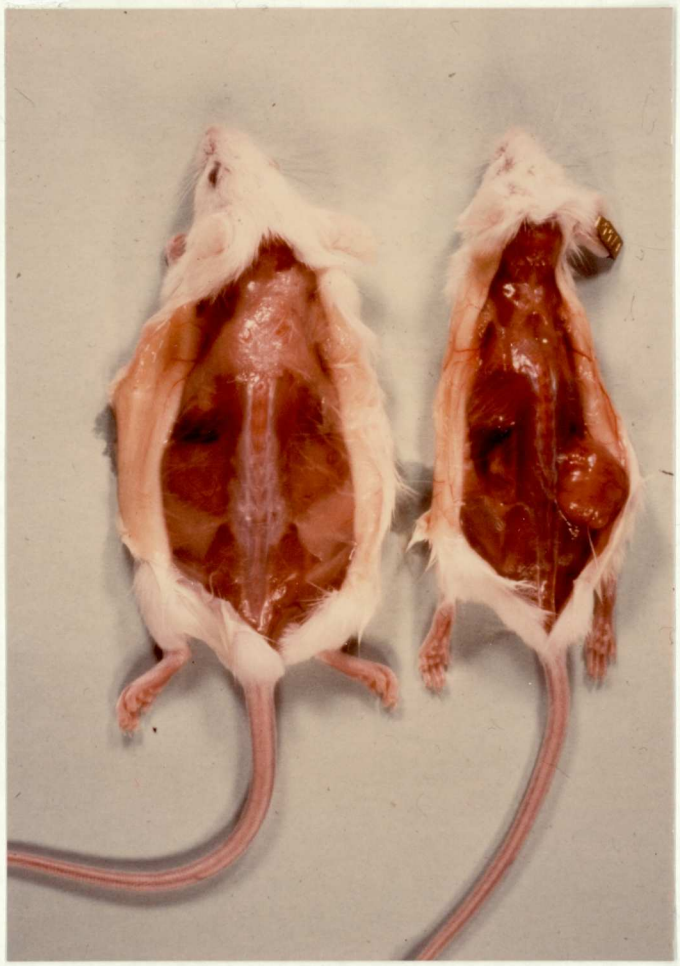


Figure D.2.5

The effects of the MAC 16 tumour on the growth and fat content of NMR I mice. Both groups of mice consumed equal amounts of food (Figure D.2.2), but the mouse on the right-hand side had a fragment of the MAC 16 tumour implanted 28 days prior to the photograph being taken.

Table D.2.3 BODY COMPOSITION OF NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

Days after implantation with tumour	Wet Weight of Carcass (grams) ± SEM	Dry Weight of Carcass (grams) ± SEM	% Water Content ± SEM	Fat Content (grams) ± SEM
7	31.95±0.83	10.1±0.73	66.5±0.76	1.28±0.14
14	31.57±1.13	10.5±0.67	66.0±1.51	1.25±0.29*
21	24.48±1.40*	8.0±1.24*	68.4±2.2	0.51±0.11**
28	24.33±0.86*	7.7±0.81*	67.3±1.71	0.25±0.08**

Table D.2.4 BODY COMPOSITION OF NON TUMOUR-BEARING NMR I MICE

Day of Study	Wet Weight of Carcass (grams) ± SEM	Dry Weight of Carcass (grams) ± SEM	% Water Content ± SEM	Fat Content (grams) ± SEM
7	31.48±1.51	10.2±0.39	67.3±1.51	1.88±0.31
14	32.73±0.96	11.2±0.93	65.3±3.70	1.96±0.22
21	32.94±1.36	11.1±0.88	66.0±2.83	1.74±0.23
28	33.83	10.5	68.7	1.53

* $p<0.05$ from non tumour-bearing groups of mice

** $p<0.002$ from non tumour-bearing groups of mice and tumour-bearing group of mice on day 7

Figure D.2.6 CHANGES IN WET CARCASS WEIGHT OF NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (▲) AND NON TUMOUR-BEARING MICE (●)

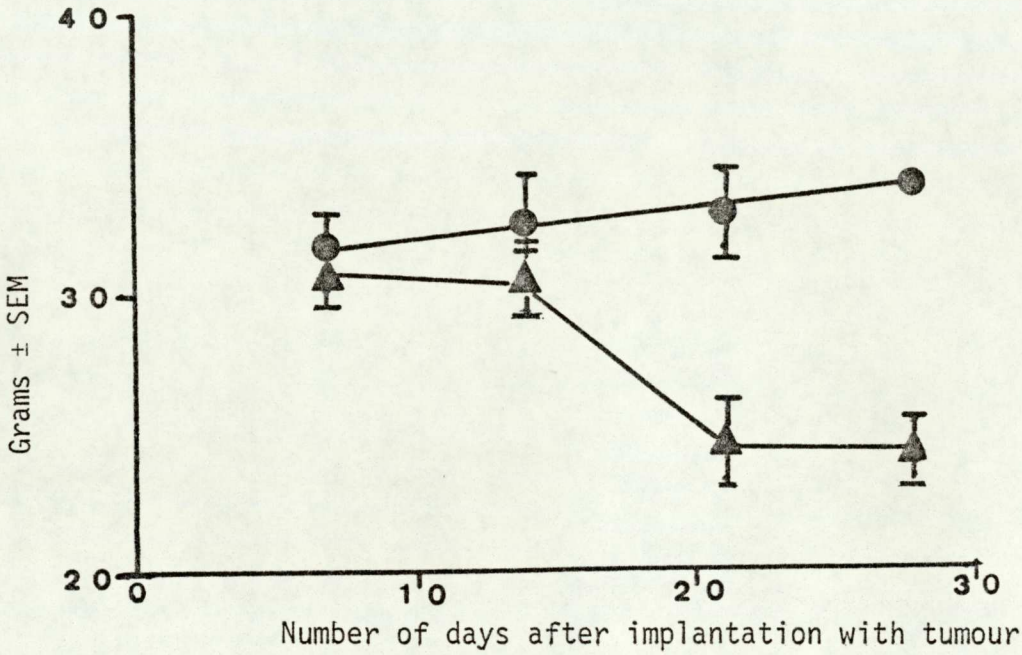


Figure D.2.7 CHANGES IN DRY CARCASS WEIGHT OF NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (▲) AND NON TUMOUR-BEARING MICE (●)

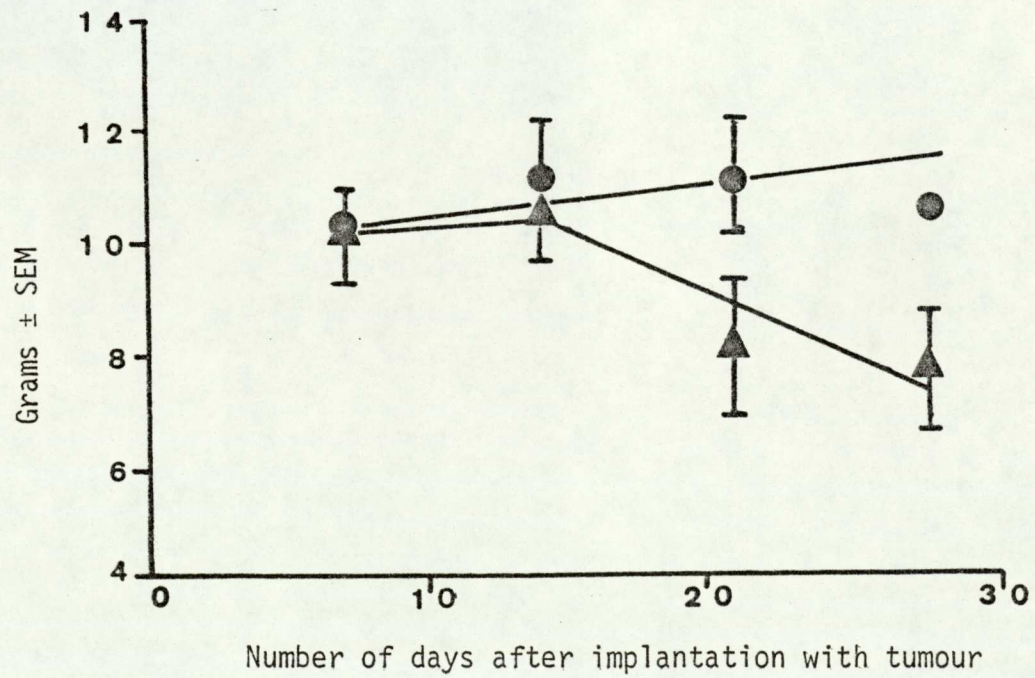


Figure D.2.8 CHANGES IN PERCENTAGE WATER CONTENT OF NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (▲) AND NON TUMOUR-BEARING MICE (●)

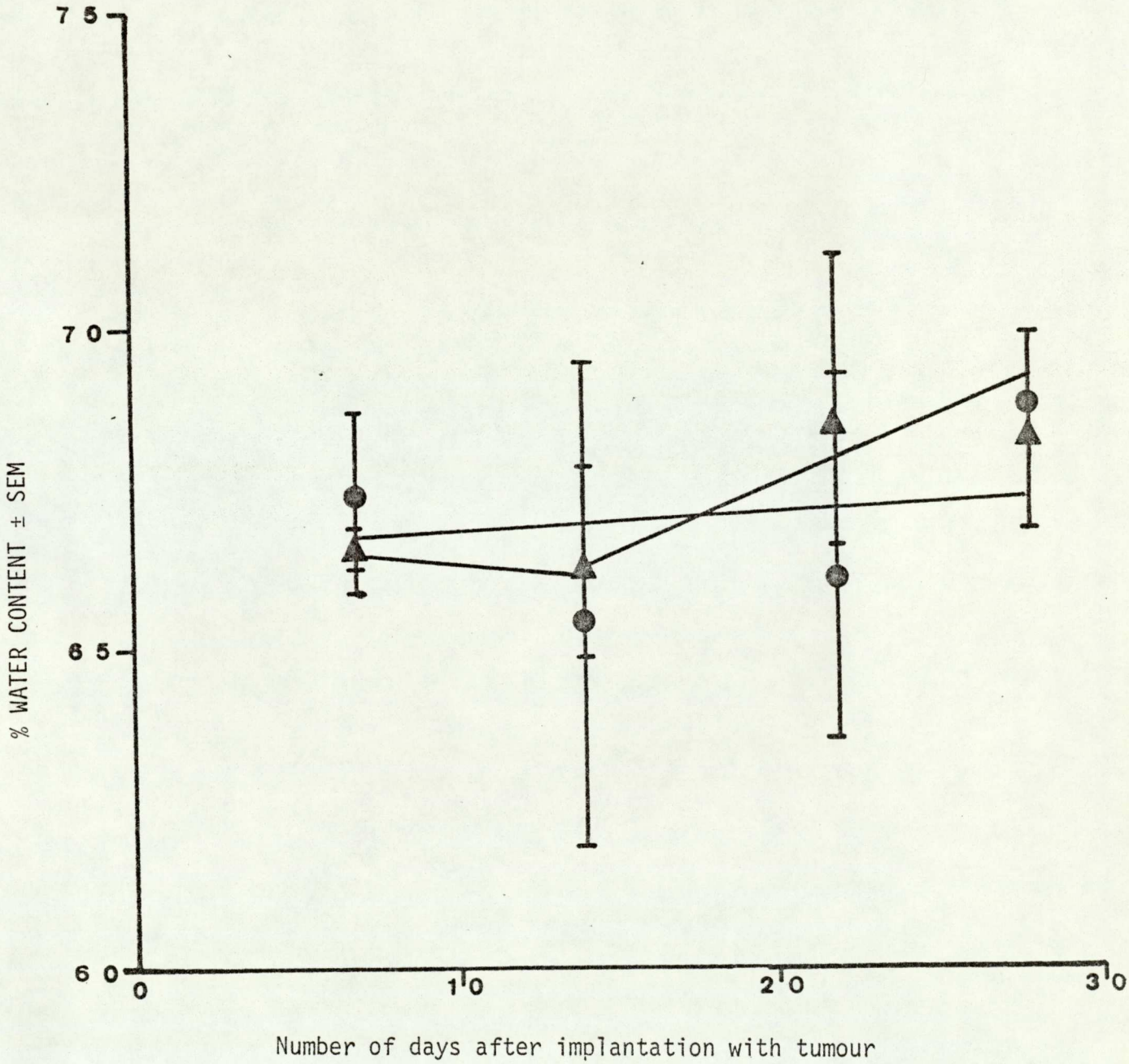
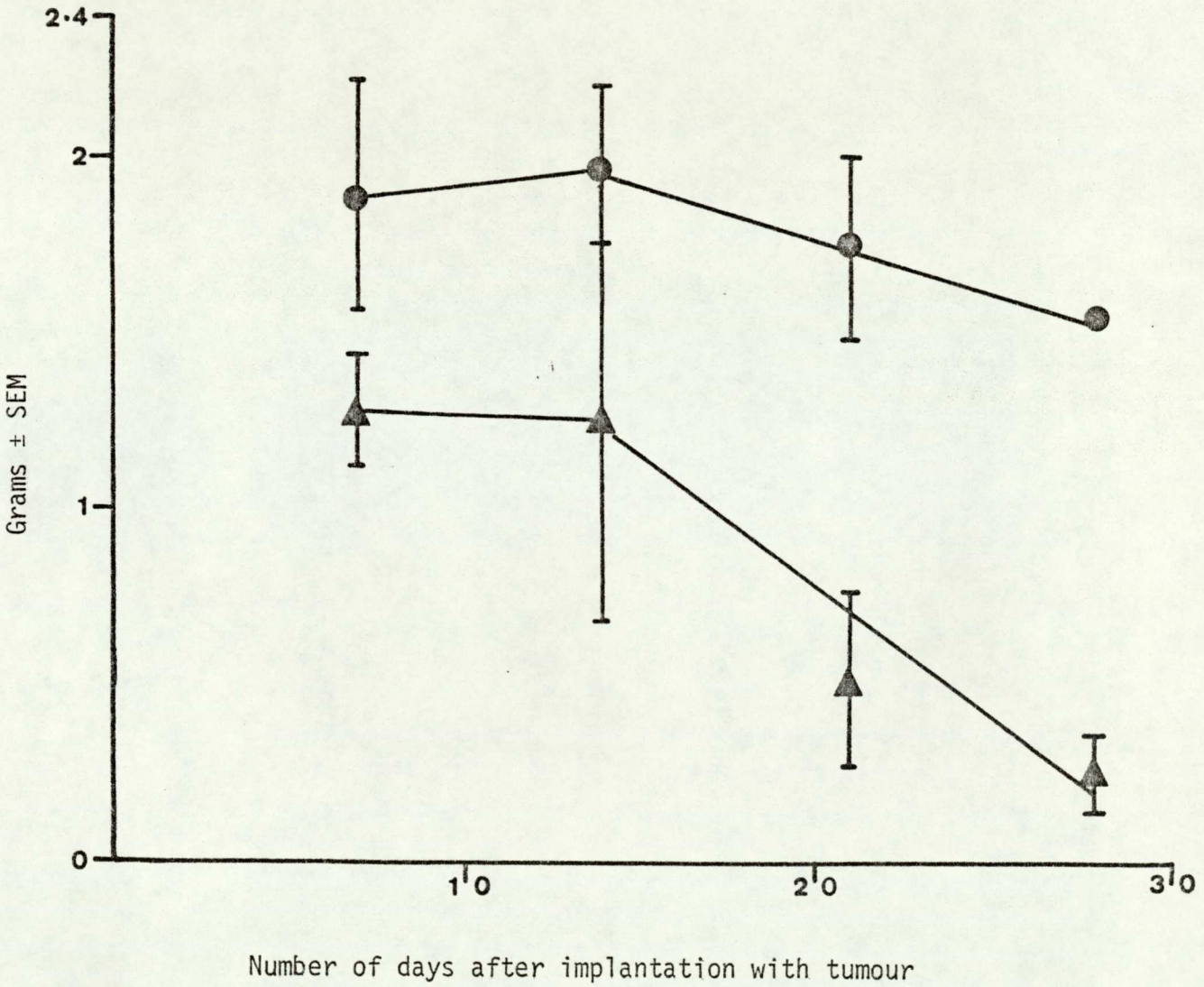


Figure D.2.9 CHANGES IN TOTAL CARCASS FAT CONTENT OF NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (▲) AND NON TUMOUR-BEARING MICE (●)



reduction in the total carcass fat content of tumour-bearing animals from day 14 onwards ($p<0.05$), and this reduction was directly related to the size of the tumour (figure D.2.10). The relationship between total carcass fat content and tumour weight is shown in figure D.2.10. There were no significant differences in the water content of the carcasses of tumour-bearing and non tumour-bearing animals. The mean weights of the thigh and gastrocnemius muscles from tumour-bearing and non tumour-bearing mice are shown in Tables D.2.5 and D.2.6 respectively, these were variable and it was not possible to draw any conclusions from them. However, although muscle mass was decreased in tumour-bearing animals, the percentage contribution towards the total body mass remained constant.

D.2.4 **Blood glucose levels**

Blood glucose levels for both groups of mice are shown in Table D.2.7 and changes in these levels over the duration of the study are shown in figure D.2.11. Levels fell in the tumour-bearing group after day 14, and were significantly lower than levels measured in non tumour-bearing mice by day 21 ($p<0.05$).

D.2.5 **Plasma insulin levels**

Levels of plasma insulin for tumour-bearing and non tumour-bearing mice are shown in Table D.2.8 and changes in these levels during the study are shown in figure D.2.12. The level of insulin in the plasma of the tumour-bearing mice was lower than those found in the non tumour-bearing mice and fell with increase in tumour size. The presence of tumour results in a fall in plasma insulin levels

Figure D.2.10 THE RELATIONSHIP BETWEEN TOTAL CARCASS FAT CONTENT AND TUMOUR WEIGHT IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

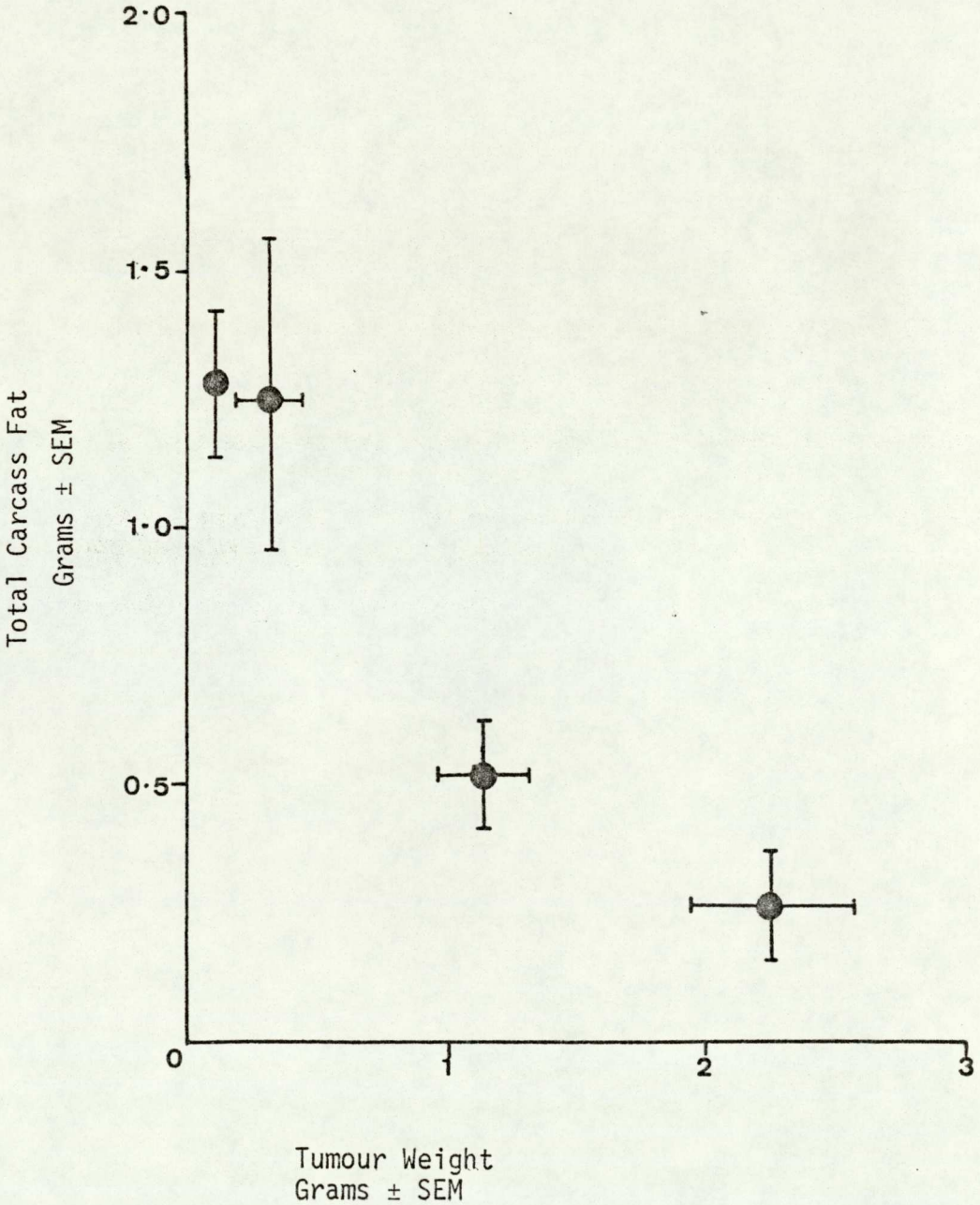


Table D.2.5 MUSCLE WEIGHTS OF NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

Days after implantation with tumour	Thigh Muscle mg		% Total Body Weight	Gastrocnemius Muscle mg		% Total Body Weight
	Wet Weight	Dry Weight		Wet Weight	Dry Weight	
7	157.6	40.5	0.61	186.3	46.8	0.72
14	154.4	37.8	0.61	157.4	39.8	0.68
21	143.8	36.9	0.73	160.9	37.5	0.88
28	135.2	32.9	0.63	201.0	43.5	0.94

Table D.2.6 MUSCLE WEIGHTS OF NON TUMOUR-BEARING NMR I MICE

Day of Study	Thigh Muscle mg		% Total Body Weight	Gastrocnemius Muscle mg		% Total Body Weight
	Wet Weight	Dry Weight		Wet Weight	Dry Weight	
7	178.0	43.8	0.68	155.7	38.9	0.59
14	196.5	52.5	0.64	142.1	38.0	0.49
21	229.5	69.4	0.77	180.9	44.9	0.61
28	221.2	63.5	0.70	270.6	68.1	0.85

Table D.2.7 BLOOD GLUCOSE LEVELS IN NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	mg/100 ml blood ± SEM	
	Non tumour-bearing mice	Tumour-bearing mice
7	136.3±4.97	115.7±8.75
14	152.0±9.83	141.0±12.60
21	129.3±13.85	111.9±7.60*
28	152.2±15.4	103.5±12.7*

* $p<0.05$ from non tumour-bearing mice

Table D.2.8 PLASMA INSULIN LEVELS IN NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	ng/ml plasma	
	Non tumour-bearing mice	Tumour-bearing mice
7	1.72	1.549
14	1.61	0.850
21	5.78	0.482
28	3.31	0.476

Table D.2.9 BLOOD GLYCEROL LEVELS IN NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	µM ± SEM	
	Non tumour-bearing mice	Tumour-bearing mice
7	107.1±25.8	107.8±20.6
14	139.9±27.1	115.0±11.5
21	118.0±14.4	130.1±15.1
28	132.0±16.3	155.0±16.5

Figure D.2.11 CHANGES IN BLOOD GLUCOSE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (*) AND NON TUMOUR-BEARING MICE (●)

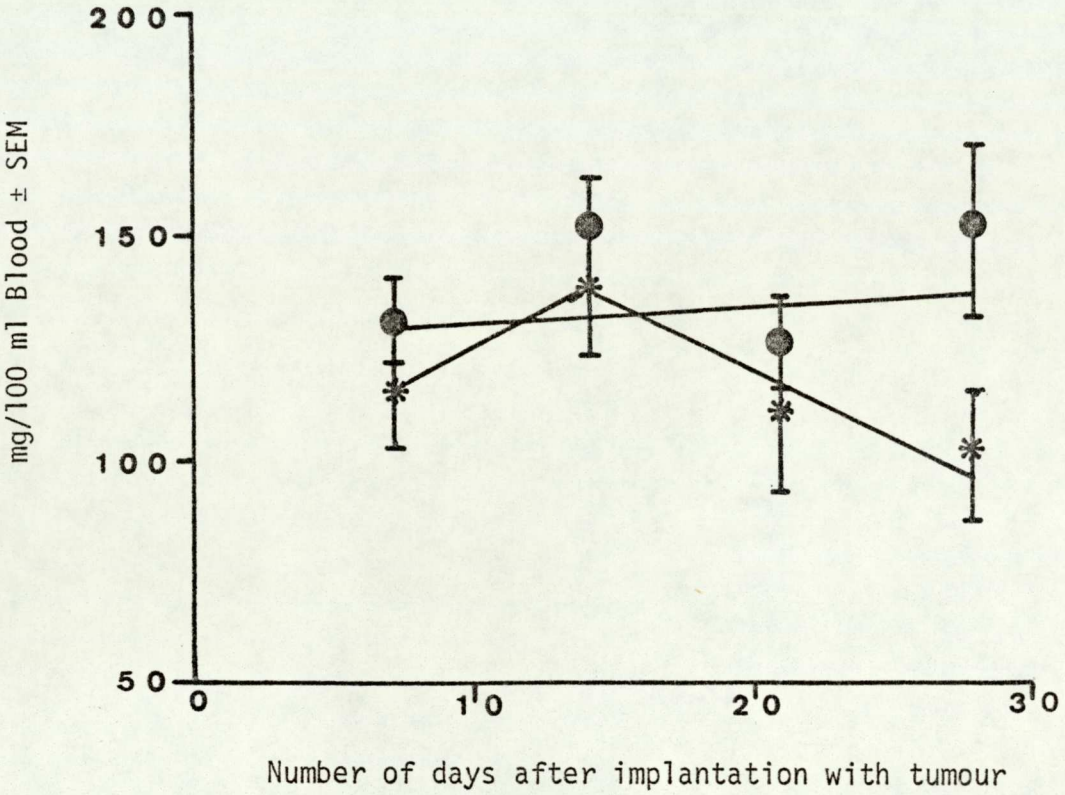
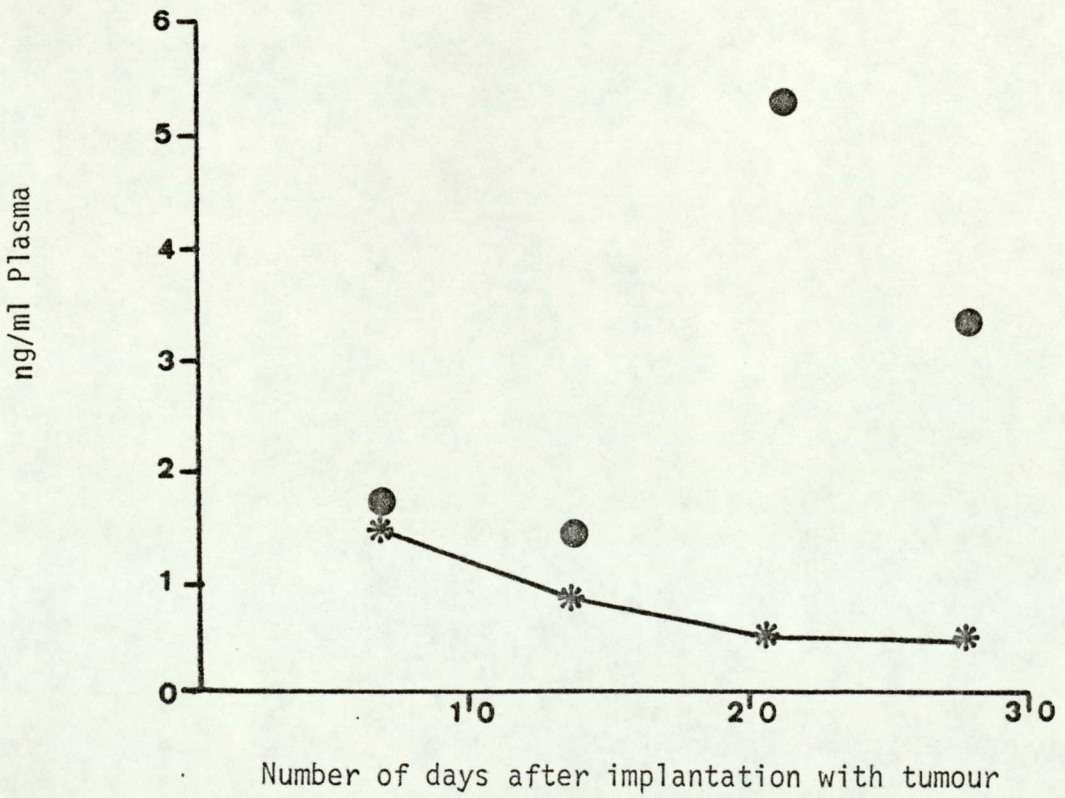


Figure D.2.12 CHANGES IN PLASMA INSULIN LEVELS OF NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (*) AND NON TUMOUR-BEARING MICE (●)



which could explain the catabolic effects seen in cachexia, especially since insulin is known to have a regulatory effect on metabolism (Moley et al, 1985).

D.2.6 Blood glycerol levels

These are shown in Table D.2.9 for both tumour-bearing and non tumour-bearing mice and changes in blood glycerol levels are shown in figure D.2.13. Levels of blood glycerol increased in both groups of mice over the period of the study. Although the levels of glycerol were higher in the tumour-bearing mice after day 14, these differences were not significant.

D.2.7 Blood lactate levels

Levels of lactate in the blood of tumour-bearing and non tumour-bearing mice are shown in Table D.2.10 and changes in these levels are shown in figure D.2.14. The levels of lactate were higher initially in the tumour-bearing mice on day 7, but decreased with increasing tumour size, becoming similar to the levels found in non tumour-bearing mice by day 21.

D.2.8 Plasma free fatty acid levels

Plasma free fatty acid levels for both groups of mice are shown in Table D.2.11, and figure D.2.15 shows changes in these levels over the period of the study. Levels of plasma free fatty acids rose initially in tumour-bearing mice to a level of 0.947 mM by day 14 and then decreased to a level of 0.283 mM by day 28.

Table D.2.10 BLOOD LACTATE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	mM ± SEM	
	Non tumour-bearing mice	Tumour-bearing mice
7	4.01±0.41	7.42
14	4.97±0.73	3.61±0.81
21	4.78±1.03	5.09±0.66
28	6.53	3.45

Table D.2.11 PLASMA FREE FATTY ACID LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	mM ± SEM	
	Non tumour-bearing mice	Tumour-bearing mice
7	0.52±0.23	0.8±0.46
14	0.84±0.25	0.95±0.28
21	0.72±0.21	0.61±0.19
28	0.43	0.28±0.06

Table D.2.12 BLOOD PYRUVATE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	mM ± SEM	
	Non tumour-bearing mice	Tumour-bearing mice
7	0.129±0.027	0.114
14	0.118±0.011	0.094±0.013
21	0.092±0.029	0.097±0.004

Figure D.2.13 CHANGES IN BLOOD GLYCEROL LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (*) AND NON TUMOUR-BEARING MICE (●)

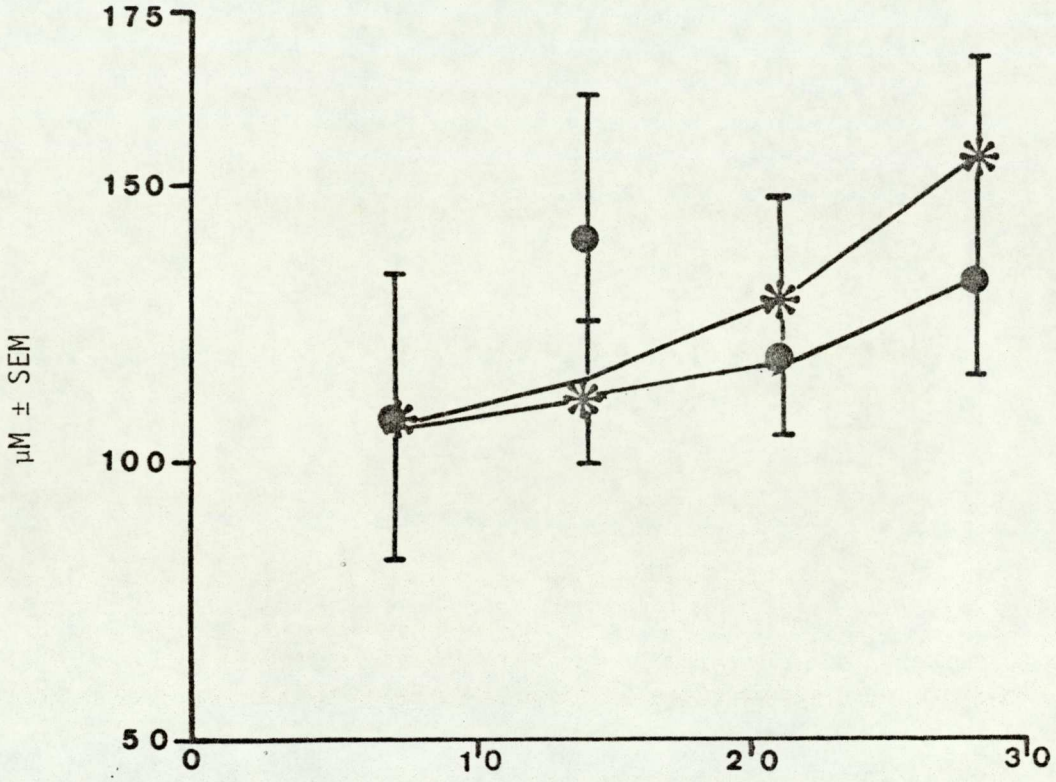


Figure D.2.14 CHANGES IN BLOOD LACTATE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (*) AND NON TUMOUR-BEARING MICE (●)

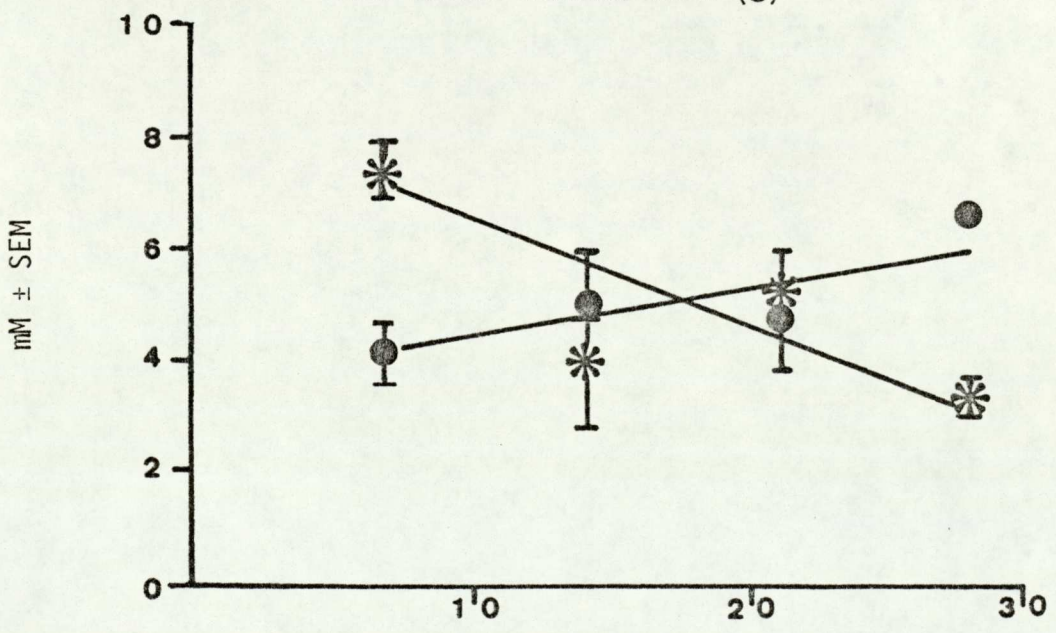


Figure D.2.15 CHANGES IN PLASMA FREE FATTY ACID LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (✱) AND NON TUMOUR-BEARING MICE (●)

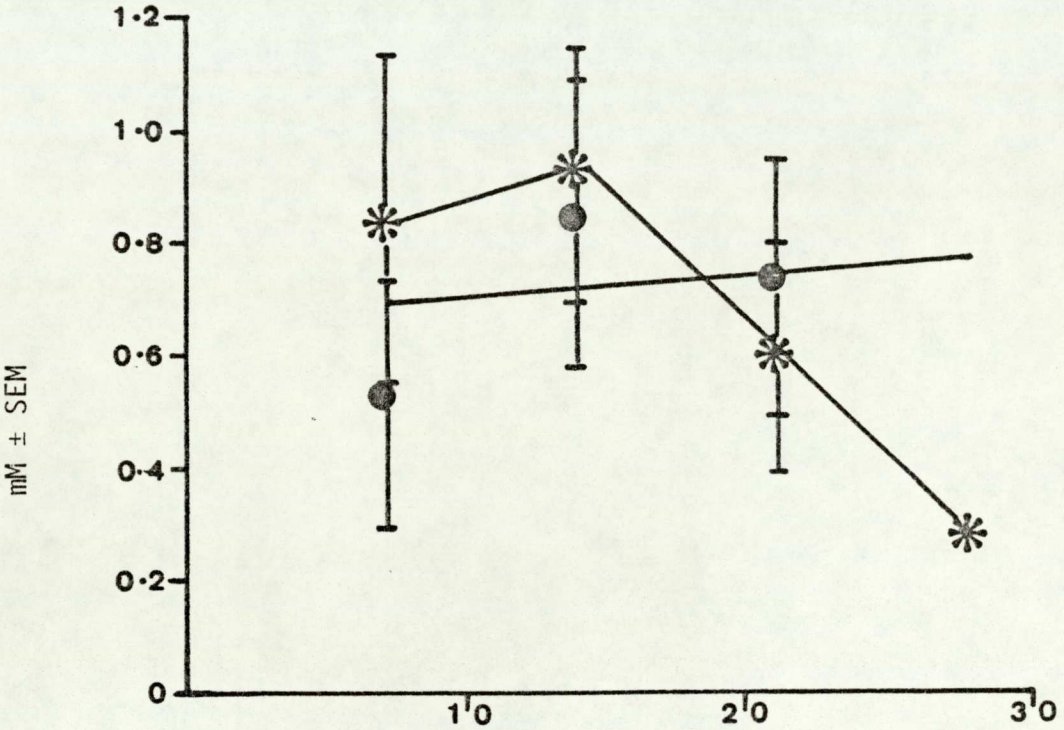
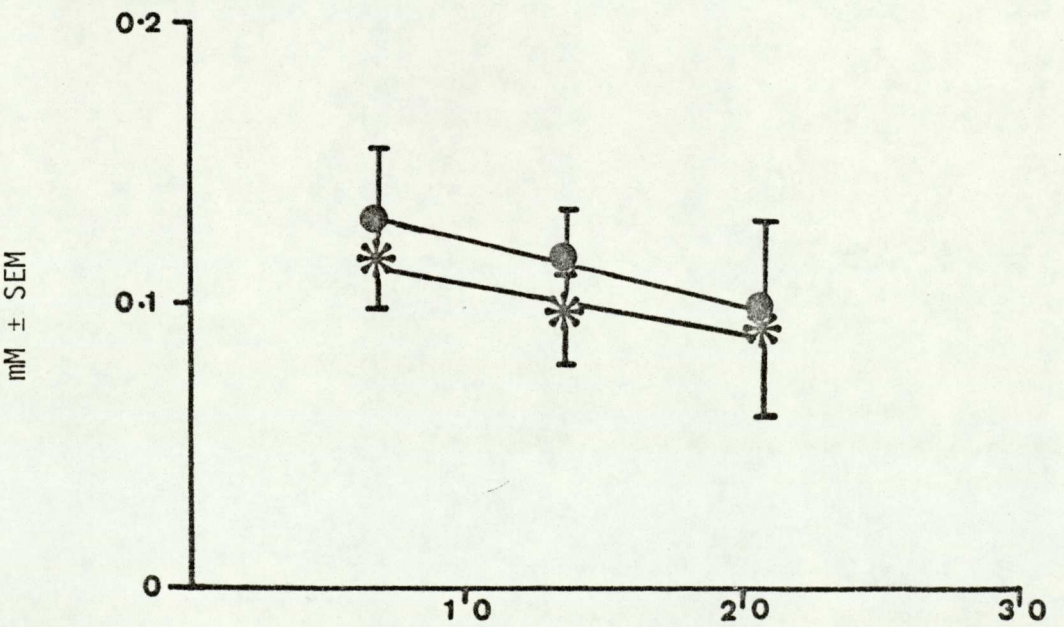


Figure D.2.16 CHANGES IN BLOOD PYRUVATE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (✱) AND NON TUMOUR-BEARING MICE (●)



D.2.9 Blood pyruvate levels

These are shown in Table D.2.12, and changes in the levels of this metabolite are shown in figure D.2.16. There was little alteration in the blood levels of pyruvate in tumour-bearing mice and no significant differences were found in the levels between tumour-bearing and non tumour-bearing mice.

D.2.10 Blood acetoacetate and 3-hydroxybutyrate levels

The levels of the ketone bodies acetoacetate and 3-hydroxybutyrate measured in the blood of tumour-bearing and non tumour-bearing mice are shown in Tables D.2.13 and D.2.14, respectively. Changes in the levels of these metabolites during the study are shown in figure D.2.17 for acetoacetate and figure D.2.18 for 3-hydroxybutyrate. There was very little change in the levels of these metabolites during the study and levels were similar in both tumour-bearing and non tumour-bearing groups of mice. Despite the significant weight loss seen in the tumour-bearing mice in this study, there appears to a complete absence of ketosis in these animals, and this is in agreement with the findings of Conyers and others (1979a) who made the observation that ketosis was an uncommon phenomenon in cancer cachexia.

D.2.11 Blood carnitine levels

These were measured in both tumour-bearing and non tumour-bearing mice, and the mean concentration found in the tumour-bearing groups was 13.8±3.0 nmoles/ml of blood compared with 7.8±1.8 nmoles/ml of blood in the non tumour-bearing

Table D.2.13 BLOOD ACETOACETATE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	mM ± SEM	
	Non tumour-bearing mice	Tumour-bearing mice
7	0.95±0.019	0.047±0.005
14	0.067±0.008	0.070±0.017
21	0.056±0.008	0.052±0.016
28	0.062±0.008	0.057±0.015

Table D.2.14 BLOOD 3-HYDROXYBUTYRATE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

Days after implantation with tumour	mM ± SEM	
	Non tumour-bearing mice	Tumour-bearing mice
7	0.147±0.019	0.074±0.009
14	0.054±0.001	0.067±0.013
21	0.064±0.011	0.092±0.019
28	0.044±0.004	0.053±0.006

Figure D.2.17 CHANGES IN BLOOD ACETOACETATE LEVELS IN NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (✻) AND NON TUMOUR-BEARING MICE (●)

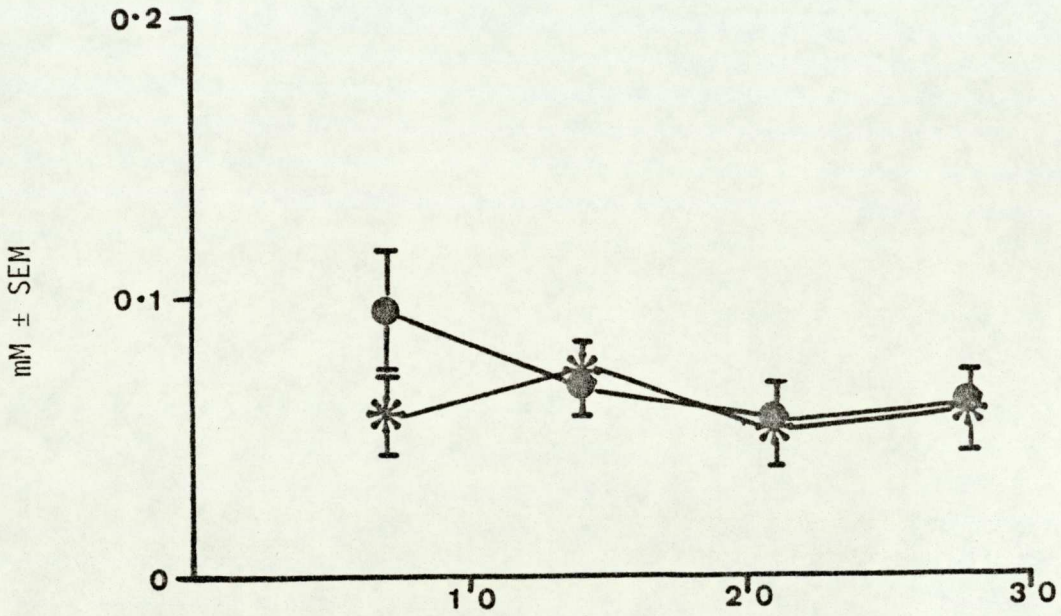
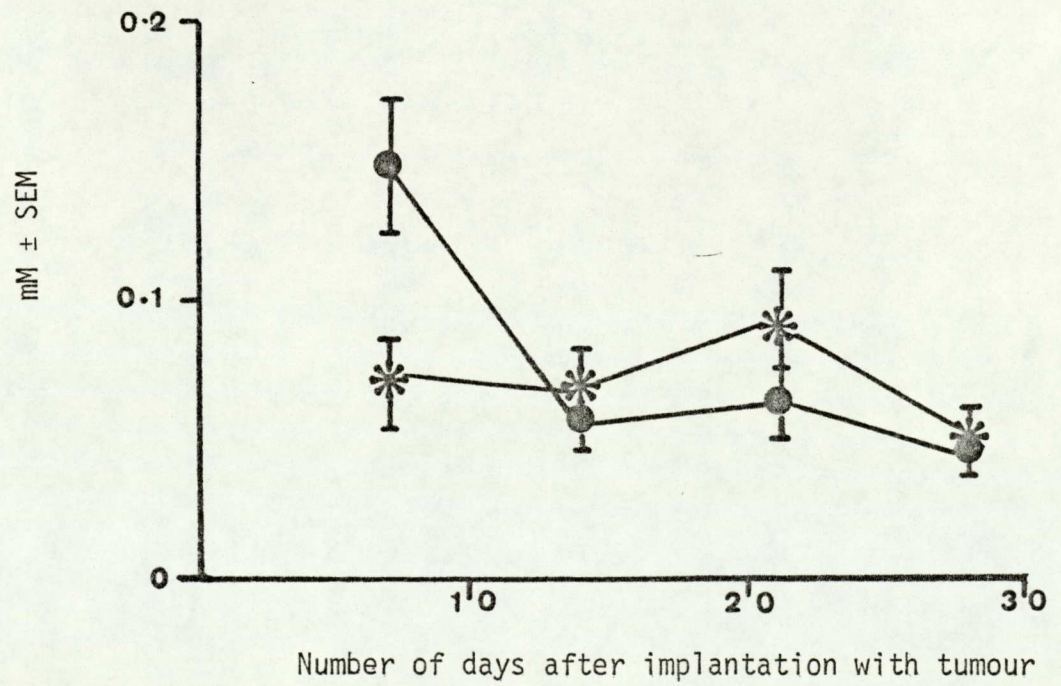


Figure D.2.18 CHANGES IN BLOOD 3-HYDROXYBUTYRATE LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (✻) AND NON TUMOUR-BEARING MICE (●)



group; however, this difference was not significant. Carnitine is required for the transport of fatty acyl-CoA across the mitochondrial membranes (figure A.3.9) into the mitochondrial matrix where β-oxidation can then proceed, and the level of this metabolite reflects the rate of β-oxidation and lipolysis, which is high in the tumour-bearing state.

D.2.12 **Amino acid profiles**

These were measured in pooled plasma samples from tumour-bearing and non tumour-bearing groups of mice on day 21 and day 28 of the study, and the results are shown in Table D.2.15 and are displayed in histogram form in figures D.2.19 and D.2.20. Levels of taurine, aspartic acid and histidine were similar in both tumour-bearing and non tumour-bearing mice. Levels of all the other amino acids measured were reduced in the tumour-bearing groups of mice. The presence of tumour was therefore found to influence plasma amino acid levels.

Table D.2.15 PLASMA AMINO ACID LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

	Day 21 nMole/ml Plasma		Day 28	
AMINO ACID	Non tumour-bearing mice	Tumour-bearing mice	Non tumour-bearing mice	Tumour-bearing mice
Taurine	280	260	370	360
Aspartic acid	7	6	8	6
Threonine	190	98	200	120
Serine	150	84	150	100
Glutamic acid / Asparagine	98	82	120	72
Glutamine	240	190	230	180
Glycine	300	170	330	200
Alanine	360	250	530	320
Citrulline	61	31	68	37
Valine	280	140	340	190
Methionine	70	42	77	45
Isoleucine	120	66	160	84
Leucine	190	110	260	130
Tyrosine	89	45	160	63
Phenylalanine	86	58	110	86
Ornithine	89	35	90	87
Lysine	310	170	280	190
Tryptophan	-	50	160	110
Histidine	89	72	95	81
Arginine	210	85	180	51

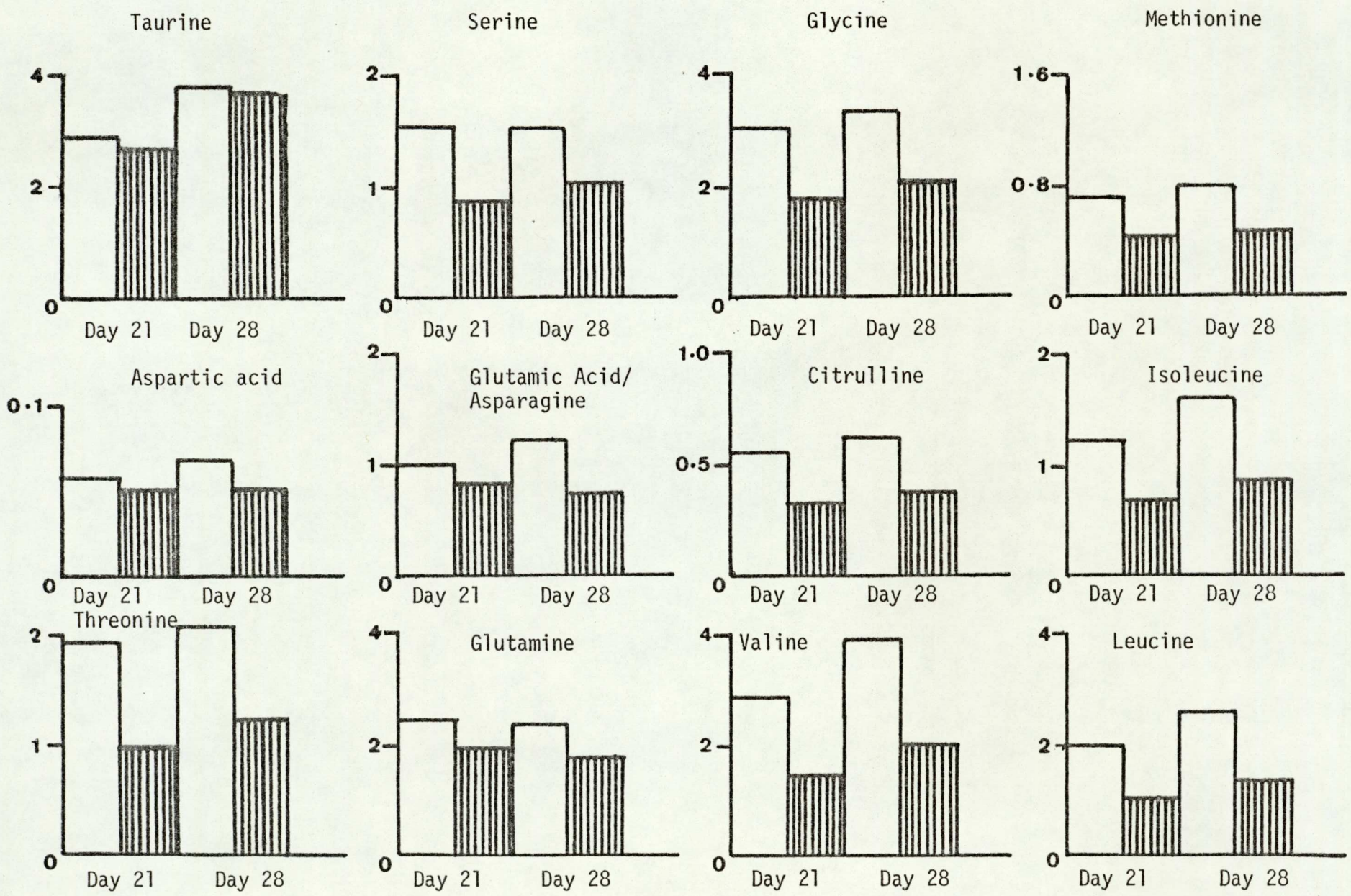


Figure D.2.19 PLASMA AMINO ACID LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (HATCHED BOXES) AND NON TUMOUR-BEARING MICE (OPEN BOXES)

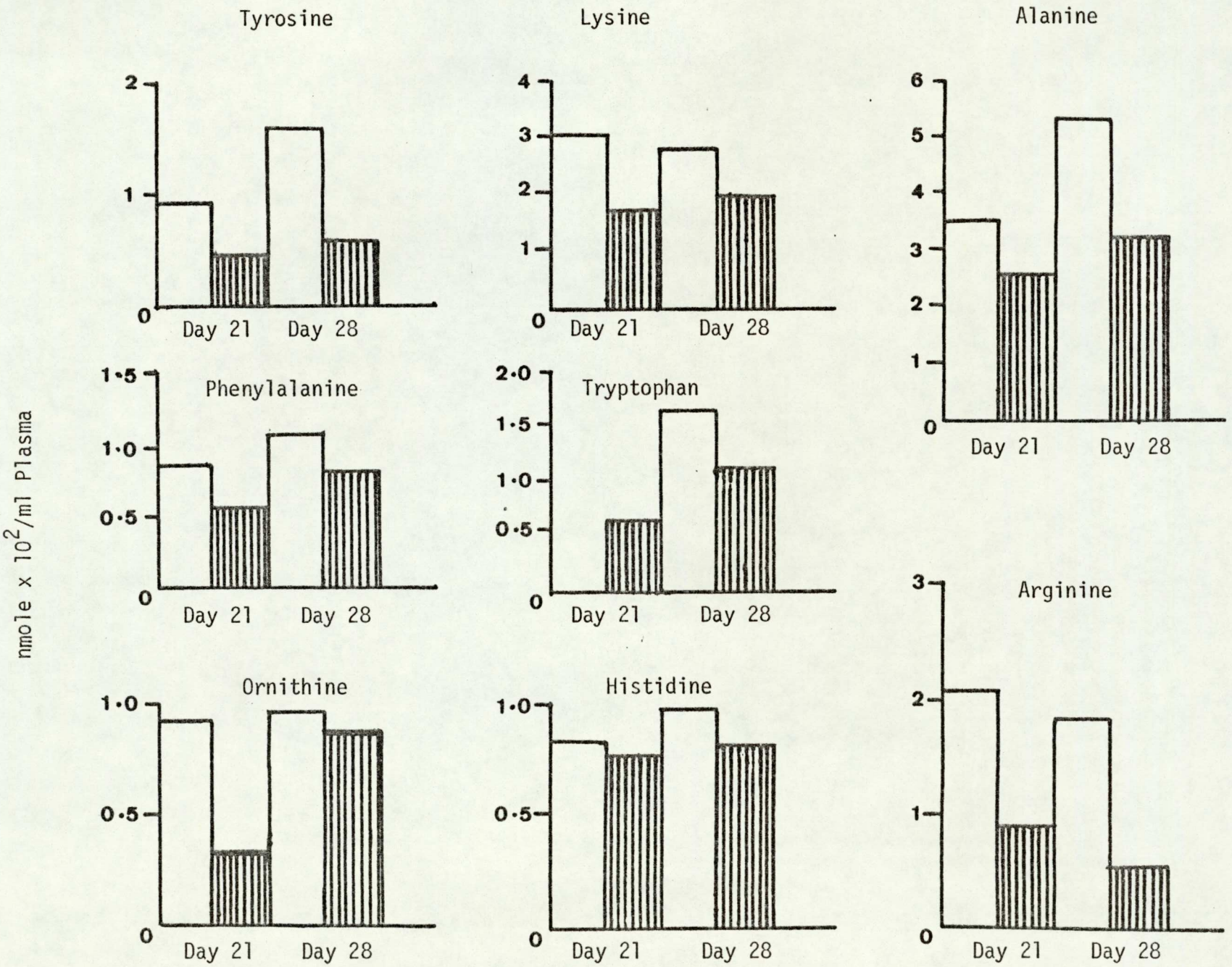


Figure D.2.20 PLASMA AMINO ACID LEVELS IN NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (HATCHED BOXES) AND NON TUMOUR-BEARING MICE (OPEN BOXES)

D.3 Diet Studies

The effects of feeding diets with increasing amounts of energy supplied from fat in the form of medium chain triglyceride (MCT) were studied in groups of NMRI mice implanted with the MAC 16 adenocarcinoma and in tumour-free mice.

D.3.1 Total body weight

Changes in total body weight over the duration of the studies for each of the diet treatment groups are shown in Tables D.3.1a and D.3.1b for female NMRI mice, and Tables D.3.2a, D.3.2b, D.3.3a and D.3.3b for male NMRI mice. The total body weights shown in Tables D.3.2a and D.3.2b are those from a single experiment, whilst those in Tables D.3.3a and D.3.3b are the aggregated results from several experiments using male NMRI mice. They are shown separately because the animals in the first experiment were of a much lower starting body weight than those used in the subsequent experiments. The overall average weight change for each diet treatment group are shown in Tables D.3.4 and D.3.5 and displayed in histogram form in Figures D.3.1 and D.3.2 for female and male mice respectively. The greatest weight loss occurred in tumour-bearing mice fed normal pelleted diet, the average overall weight loss being 7.54 grams for female mice, and 5.83 ± 1.07 grams for male mice. In female mice this represents as much as 33% of the total body weight 28 days after implantation with tumour, and in males almost 20% of the total body weight 24 days after implantation with tumour when compared with age-matched controls. The lowest weight loss was seen in those tumour-bearing mice fed diets containing 80% MCT supplemented with 3-hydroxybutyrate

Table D.3.1a THE EFFECT OF DIETARY MODIFICATION ON TOTAL BODY WEIGHT OF FEMALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	MEAN TOTAL BODY WEIGHT ± SEM							
	NUMBER OF DAYS AFTER IMPLANTATION WITH TUMOUR							
A	1	4	8	9	10	11	12	13
Normal diet	21.2±0.5	21.2±0.7	20.7±0.7	20.4±0.6	20.6±0.5	20.3±0.4	19.9±0.6	20.4±0.4
52.5% MCT	21.1±1.4	21.3±1.4	20.9±1.3	20.9±1.2	20.6±1.3	20.7±1.1	20.3±1.0	19.4±1.0
52.5% MCT + 3OHB	22.4±0.7	22.1±0.6	22.3±0.5	21.5±0.7	20.8±1.0	20.9±1.1	20.6±1.2	20.6±1.0
68% MCT	21.1±0.9	20.7±0.9	20.3±0.7	19.7±0.7	19.9±0.8	20.0±0.7	19.9±0.6	20.4±0.7
68% MCT + 3OHB	2.15±0.5	20.0±0.5	21.0±0.6	20.2±0.5	19.8±0.5	20.2±0.6	20.3±0.7	20.1±0.5
B								
Normal diet	24.5±2.4	22.9±2.4	24.3±2.5	23.7±2.3	23.8±2.0	23.7±2.0	23.8±2.1	23.8±2.2
68% MCT + 3OHB	21.9±0.3	20.7±0.3	22.7±0.5	21.5±0.4	21.3±0.3	21.3±0.2	21.1±0.4	20.9±0.5

DIETARY TREATMENTS STARTED

Table D.3.1b THE EFFECT OF DIETARY MODIFICATION ON TOTAL BODY WEIGHT OF FEMALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	MEAN TOTAL BODY WEIGHT ± SEM								
	NUMBER OF DAYS AFTER IMPLANTATION WITH TUMOUR								
A	16	17	18	19	22	23	24	25	28
Normal diet	19.7±0.5	19.1±0.9	18.8±0.9	18.2±1.6	17.8±1.6	17.9±1.6	17.8±1.6	17.7±1.5	17.8±1.6
52.5% MCT	21.0±1.1	20.9±1.3	20.7±1.5	19.8±1.7	19.9±1.5	19.7±1.3	19.4±1.3	18.5±1.4	17.4±1.1
52.5% MCT + 3OHB	20.6±0.9	20.3±0.9	20.0±1.1	19.9±1.6	20.4±1.3	19.7±1.4	19.3±1.3	19.2±1.4	17.8±1.1
68% MCT	21.2±0.6	20.8±0.7	20.5±0.7	19.5±1.3	19.4±1.1	19.4±1.4	19.3±1.4	19.5±1.4	19.0±1.3
68% MCT + 3OHB	20.3±0.5	19.9±0.6	19.7±0.6	19.2±0.9	18.8±0.9	19.0±0.8	18.9±0.7	19.0±0.7	17.9±0.7
B									
Normal diet	23.5±2.0	23.3±2.0	23.4±2.1	23.3±2.1	23.6±2.2	23.9±2.2	23.6±2.2	23.9±2.1	24.1±2.4
68% MCT + 3OHB	20.8±0.5	20.6±0.4	20.6±0.4	20.8±0.3	20.7±0.4	20.8±0.3	20.7±0.5	20.6±0.3	20.1±0.4

Table D.3.2a THE EFFECT OF DIETARY MODIFICATION ON TOTAL BODY WEIGHT OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B). (LOWER STARTING BODY WEIGHTS)

DIETARY TREATMENT	MEAN TOTAL BODY WEIGHT ± SEM								
	NUMBER OF DAYS AFTER IMPLANTATION WITH TUMOUR								
A	1	2	5	8	9	10	11	12	15
Normal diet	25.9±0.5	26.4±0.3	26.8±0.4	27.5±0.2	27.8±0.2	27.7±0.2	28.1±0.3	28.1±0.3	27.6±0.2
68% MCT	26.0±0.7	26.9±0.8	26.5±0.8	25.9±0.7	27.2±0.8	27.2±0.9	27.2±1.0	27.6±1.0	26.4±1.0
68% MCT +3OHB	25.4±1.1	26.6±0.9	26.7±0.5	25.9±0.5	27.2±0.5	26.9±1.3	27.1±1.3	27.2±1.4	26.2±1.7
80% MCT	25.9±0.5	27.1±0.8	27.3±0.7	26.6±0.7	27.8±0.5	27.4±0.5	27.7±0.7	27.8±0.6	26.5±1.9
B									
Normal diet	27.3±0.8	28.3±0.74	28.3±0.6	28.8±0.7	28.9±0.7	29.1±0.7	29.2±0.6	29.0±0.6	30.2±0.6
80% MCT	26.9±1.1	27.9±1.3	28.7±1.2	27.7±0.9	27.7±0.9	28.2±1.0	28.8±1.1	27.1±1.0	29.3±1.1

Dietary Treatments Started

Table D.3.2b THE EFFECT OF DIETARY MODIFICATION ON TOTAL BODY WEIGHT OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B). (LOWER STARTING BODY WEIGHTS)

DIETARY TREATMENT	MEAN TOTAL BODY WEIGHT ± SEM						
	NUMBER OF DAYS AFTER IMPLANTATION WITH TUMOUR						
A	16	17	18	19	22	23	24
Normal diet	27.4±0.9	26.2±1.4	25.7±1.5	25.1±1.5	23.6±2.1	23.4±1.5	23.0±1.2
68% MCT	27.5±1.0	27.4±1.0	26.8±1.4	26.0±1.5	25.0±1.8	23.7±1.8	23.7±1.7
68% MCT +3OHB	26.7±2.2	26.5±2.3	26.4±2.3	25.8±2.1	23.6±2.5	24.5±2.6	24.8±2.8
80% MCT	27.9±1.7	28.6±1.8	27.8±1.7	27.5±1.9	26.3±2.6	26.6±2.5	26.2±2.5
B							
Normal diet	30.2±0.6	29.7±0.6	30.1±0.6	30.3±0.6	31.0±0.6	30.6±0.7	30.5±0.8
80% MCT	29.6±1.2	29.6±1.1	29.9±1.1	30.2±1.4	30.9±1.3	30.7±1.2	31.1±1.1

Table D.3.3a THE EFFECT OF DIETARY MODIFICATION ON TOTAL BODY WEIGHT OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	MEAN TOTAL BODY WEIGHT ± SEM								
	NUMBER OF DAYS AFTER IMPLANTATION WITH TUMOUR								
A	1	3	5	8	9	10	11	12	15
Normal diet	31.2±0.6	31.6±0.6	31.4±0.7	31.3±0.6	31.4±0.6	31.5±0.6	31.0±0.5	30.5±0.6	30.3±0.6
Normal diet + 20% sucrose	33.2±0.3	33.1±0.5	32.4±0.4	32.3±0.3	32.2±0.5	32.5±0.5	33.0±0.8	33.1±0.8	33.3±0.6
68% MCT + 3OHB	31.6±0.5	31.7±0.6	31.7±0.5	30.8±0.5	30.7±0.5	30.7±0.6	30.9±0.6	29.9±1.7	30.3±0.8
80% MCT	32.1±0.4	31.6±0.4	32.1±0.5	30.5±0.5	30.1±0.5	30.3±0.4	30.6±0.5	30.8±0.6	30.8±0.5
80% MCT + 3OHB	32.4±0.5	31.9±0.4	32.3±0.5	32.1±0.4	31.7±0.4	30.7±0.5	31.6±0.6	31.8±0.6	32.0±0.9
B									
Normal diet	32.1±0.5	32.3±0.6	32.4±0.5	32.6±0.6	33.1±0.6	32.8±0.6	33.3±0.6	33.1±0.7	33.6±0.6
Normal diet + 20% sucrose	34.1±0.6	34.2±0.6	34.7±0.7	34.6±0.7	34.7±0.6	34.8±0.6	32.9±0.6	32.6±0.5	33.0±0.6
68% MCT	32.1±0.9	31.9±0.8	31.2±0.9	31.9±1.1	31.9±1.1	32.9±1.3	32.6±1.2	33.2±1.3	32.9±1.3

Dietary treatments started

Table D.3.3a continued

B

68% MCT + 3OHB	33.1±1.8	32.7±1.6	34.9±1.3	32.9±1.4	33.8±1.1	33.3±0.9	33.3±1.0	33.7±0.6	33.7±0.8
80% MCT	30.2±0.4	28.8±0.3	31.2±0.2	29.5±0.3	29.5±0.4	29.9±0.4	30.4±0.5	30.6±0.5	30.4±0.5
80% MCT + 3OHB	33.4±0.5	32.0±0.9	31.9±1.4	34.4±1.8	32.8±1.3	33.4±1.3	33.2±1.4	33.1±1.2	33.5±1.1

Dietary Treatment Started

Table D.3.3b THE EFFECT OF DIETARY MODIFICATION ON TOTAL BODY WEIGHT OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	MEAN TOTAL BODY WEIGHT ± SEM						
	NUMBER OF DAYS AFTER IMPLANTATION WITH TUMOUR						
A	16	17	18	19	22	23	24
Normal diet	30.5±0.7	30.2±0.8	27.7±1.2	26.4±1.1	27.9±1.4	27.6±1.3	26.4±1.9
Normal diet + 20% sucrose	32.9±0.7	33.2±0.7	32.8±0.7	32.6±0.7	32.8±0.7	30.7±0.9	29.1±1.3
68% MCT + 3OHB	30.2±0.9	30.2±0.9	30.3±0.8	30.4±1.4	29.8±1.5	28.8±1.3	30.3±1.7
80% MCT	30.5±0.5	29.7±0.6	29.2±0.7	29.6±0.7	29.5±1.1	30.1±0.9	29.2±1.4
80% MCT + 3OHB	30.7±0.9	31.2±0.7	31.6±0.8	31.4±0.8	30.6±0.9	28.5±1.1	27.9±1.6
B							
Normal diet	33.1±0.5	32.8±0.5	33.2±0.6	34.4±0.7	33.2±0.5	33.5±0.6	30.5±0.6
Normal diet + 20% sucrose	32.7±0.6	32.4±0.7	33.4±0.7	33.6±0.8	33.5±1.0	33.1±1.3	33.0±1.2

Table D.3.3b continued

B

68% MCT	33.1±1.1	33.8±1.3	33.4±1.1	33.6±1.2	33.8±1.3	33.0±1.2	32.8±1.1
68% MCT + 3OHB	34.1±0.6	33.9±0.7	34.1±0.4	34.9±0.7	34.4±0.4	34.3±0.3	34.9±0.5
80% MCT	30.0±1.0	29.6±1.1	30.1±1.3	30.2±1.1	30.9±1.4	30.73±1.1	31.1±1.2
80% MCT + 3OHB	33.9±1.1	33.5±1.3	33.9±1.3	33.6±1.3	34.3±1.2	33.3±1.0	34.2±1.4

Table D.3.4 THE EFFECT OF DIETARY MODIFICATION ON THE OVERALL WEIGHT CHANGE OF FEMALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND OF NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	MEAN OVERALL WEIGHT CHANGE (GRAMS) ± SEM
A	
Normal diet	-7.54
52.5% MCT	-4.61 ± 1.63
52.5% MCT + 3-Hydroxybutyrate	-6.21 ± 0.91 * **
68% MCT	-2.61 ± 1.82
68% MCT + 3-Hydroxybutyrate	-3.66 ± 1.23
B	
Normal diet	-0.19 ± 0.14 **
68% MCT + 3-Hydroxybutyrate	-2.66 ± 0.29

* $p < 0.0003$ from non tumour-bearing group of mice fed normal diet

** $p < 0.002$ from non tumour-bearing group of mice fed 68% MCT plus 3-hydroxybutyrate arginine salt

Figure D.3.1 THE EFFECT OF DIETARY MODIFICATION ON OVERALL AVERAGE WEIGHT CHANGES IN FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND IN NON TUMOUR-BEARING MICE (BLACK BOXES)

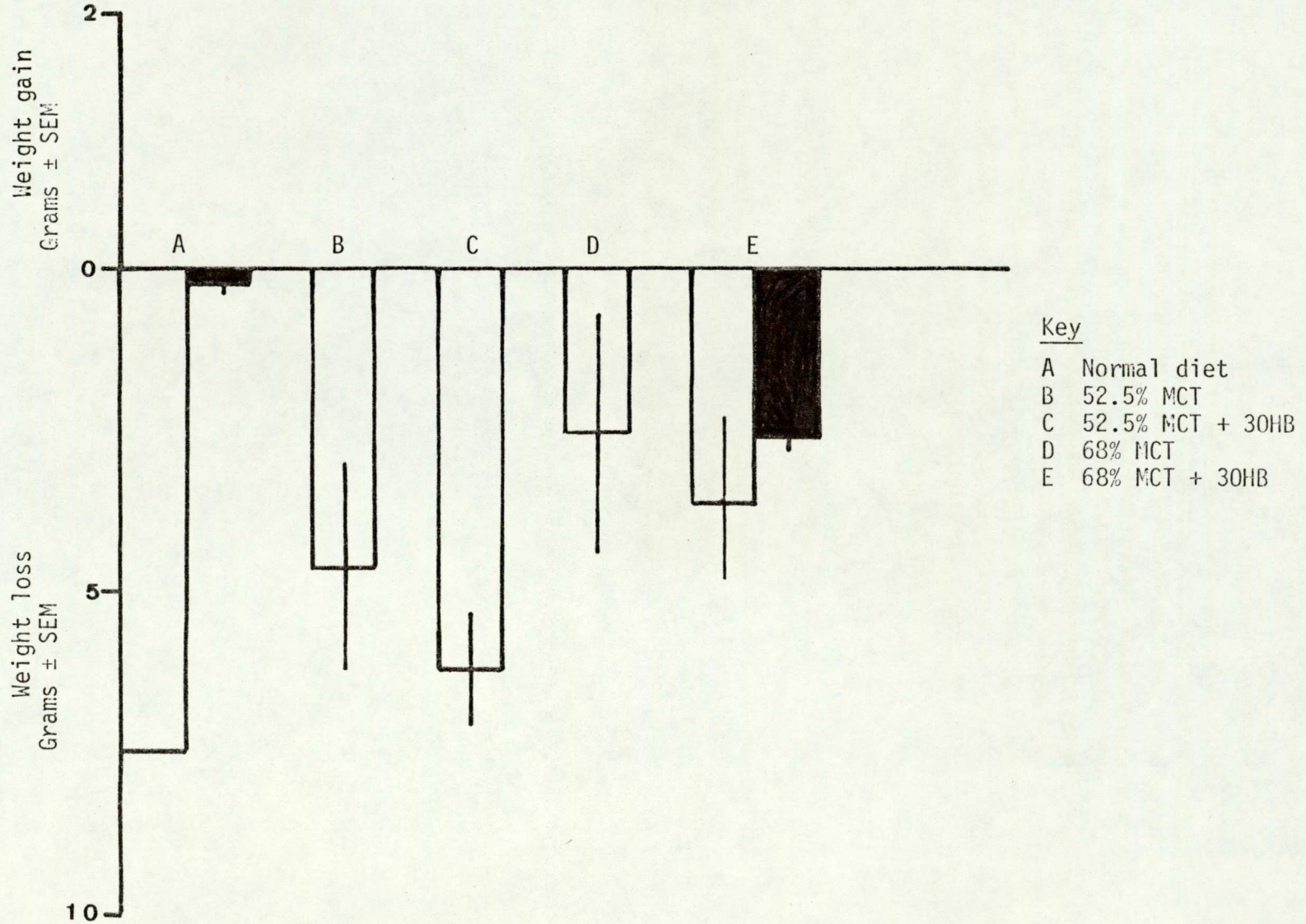


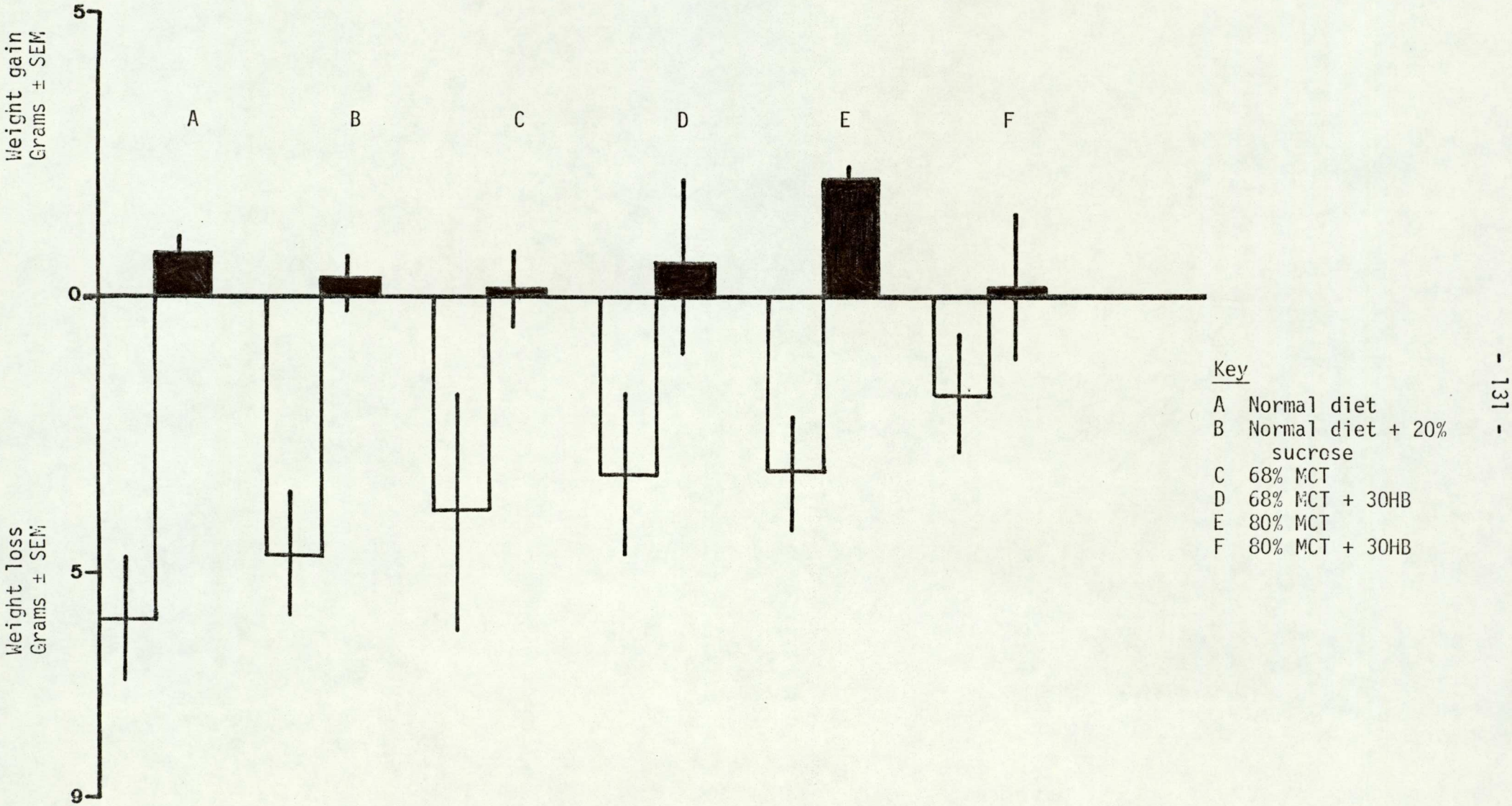
Table D.3.5 THE EFFECT OF DIETARY MODIFICATION ON THE OVERALL WEIGHT CHANGE OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND OF NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	MEAN OVERALL WEIGHT CHANGE (GRAMS) ± SEM
A	
Normal diet	-5.83 ± 1.07
Normal diet + 20% sucrose	-4.64 ± 1.11
68% MCT	-3.82 ± 2.15
68% MCT + 3-hydroxybutyrate	-3.27 ± 1.50
80% MCT	-3.19 ± 0.99
80% MCT + 3-hydroxybutyrate	-1.77 ± 1.09 * **
B	
Normal diet	+0.67 ± 0.31
Normal diet + 20% sucrose	+0.27 ± 0.58
68% MCT	+0.08 ± 0.72
68% MCT + 3-hydroxybutyrate	+0.52 ± 1.59
80% MCT	+2.08 ± 0.26
80% MCT + 3-hydroxybutyrate	+0.19 ± 1.18

* $p < 0.03$ from tumour-bearing group of mice fed normal diet

** $p < 0.02$ from non tumour-bearing group of mice fed normal diet

Figure D.3.2 THE EFFECT OF DIETARY MODIFICATION ON OVERALL AVERAGE WEIGHT CHANGES IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND IN NON TUMOUR-BEARING MICE (BLACK BOXES)



arginine salt, with an average weight loss of 1.77 ± 1.09 grams. This result was highly significant ($p < 0.03$). The average weight losses of the other tumour-bearing groups of mice fell in between these two groups in the following order:

52.5% MCT plus 3-hydroxybutyrate arginine salt

Normal diet plus 20% sucrose

52.5% MCT

68% MCT plus 3-hydroxybutyrate arginine salt

68% MCT

80% MCT

Growth of the MAC 16 tumour is accompanied by a considerable host weight loss and this weight loss may be reduced by the inclusion of increasing amounts of medium chain triglyceride in the diet and supplementation with 3-hydroxybutyrate arginine salt.

D.3.2 **Carcass weight and tumour weight**

These results are shown in Table D.3.6 and in Figures D.3.3 and D.3.4 for female mice, and Tables D.3.7 and D.3.8 and Figures D.3.5 - D.3.8 for male mice. The lowest carcass weights for female tumour-bearing mice were seen in the group fed on the normal pelleted diet, and the highest carcass weights were seen in the group fed the 68% MCT diet, although these differences were not significant. In the studies using male mice, the lowest carcass weights were seen in the groups of animals fed the normal diet. The highest carcass weights were seen in the group fed the 80% MCT diet for the study using mice with the lowest starting weights, and in the group fed the 80% MCT

Table D.3.6 THE EFFECT OF DIETARY MODIFICATION ON CARCASS WEIGHT AND TUMOUR WEIGHT OF FEMALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

DIETARY TREATMENT	CARCASS WEIGHT GRAMS ± SEM	TUMOUR WEIGHT/20 gm MOUSE GRAMS ± SEM
Normal diet	14.7	1.38
52.5% MCT	15.7 ± 1.1	1.46 ± 0.32
52.5% MCT + 3-hydroxybutyrate	16.1 ± 1.1	1.27 ± 0.33
68% MCT	17.4 ± 1.1	0.89 ± 0.22
68% MCT + 3-hydroxybutyrate	16.3 ± 1.0	1.07 ± 0.40

Table D.3.7 THE EFFECT OF DIETARY MODIFICATION ON CARCASS WEIGHT AND TUMOUR WEIGHT OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (LOWER STARTING BODY WEIGHTS)

DIETARY TREATMENT	CARCASS WEIGHT GRAMS ± SEM	TUMOUR WEIGHT/20 gm MOUSE GRAMS ± SEM
Normal diet	21.1 ± 1.9	1.01 ± 0.23
68% MCT	21.7 ± 1.6	0.96 ± 0.22
68% MCT + 3-hydroxybutyrate	23.1 ± 3.0	0.78 ± 0.28
80% MCT	24.3 ± 2.3	0.67 ± 0.19 *

* $p < 0.05$ from group of mice fed normal diet

Table D.3.8 THE EFFECT OF DIETARY MODIFICATION ON CARCASS WEIGHT AND TUMOUR WEIGHT OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

DIETARY TREATMENT A	CARCASS WEIGHT GRAMS ± SEM	TUMOUR WEIGHT/20 gm MOUSE GRAMS ± SEM
Normal diet	23.4 ± 1.8	0.96 ± 0.17
Normal diet + 20% sucrose	28.1 ± 1.4	0.89 ± 0.17
68% MCT + 3-hydroxybutyrate	23.8 ± 2.1	0.77 ± 0.23
80% MCT	25.7 ± 1.1	0.74 ± 0.09
80% MCT + 3-hydroxybutyrate	29.7 ± 1.2	0.52 ± 0.09 *

* $p < 0.01$ from group of mice fed normal diet

Figure D.3.3 THE EFFECT OF DIETARY MODIFICATION ON THE CARCASS WEIGHT OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

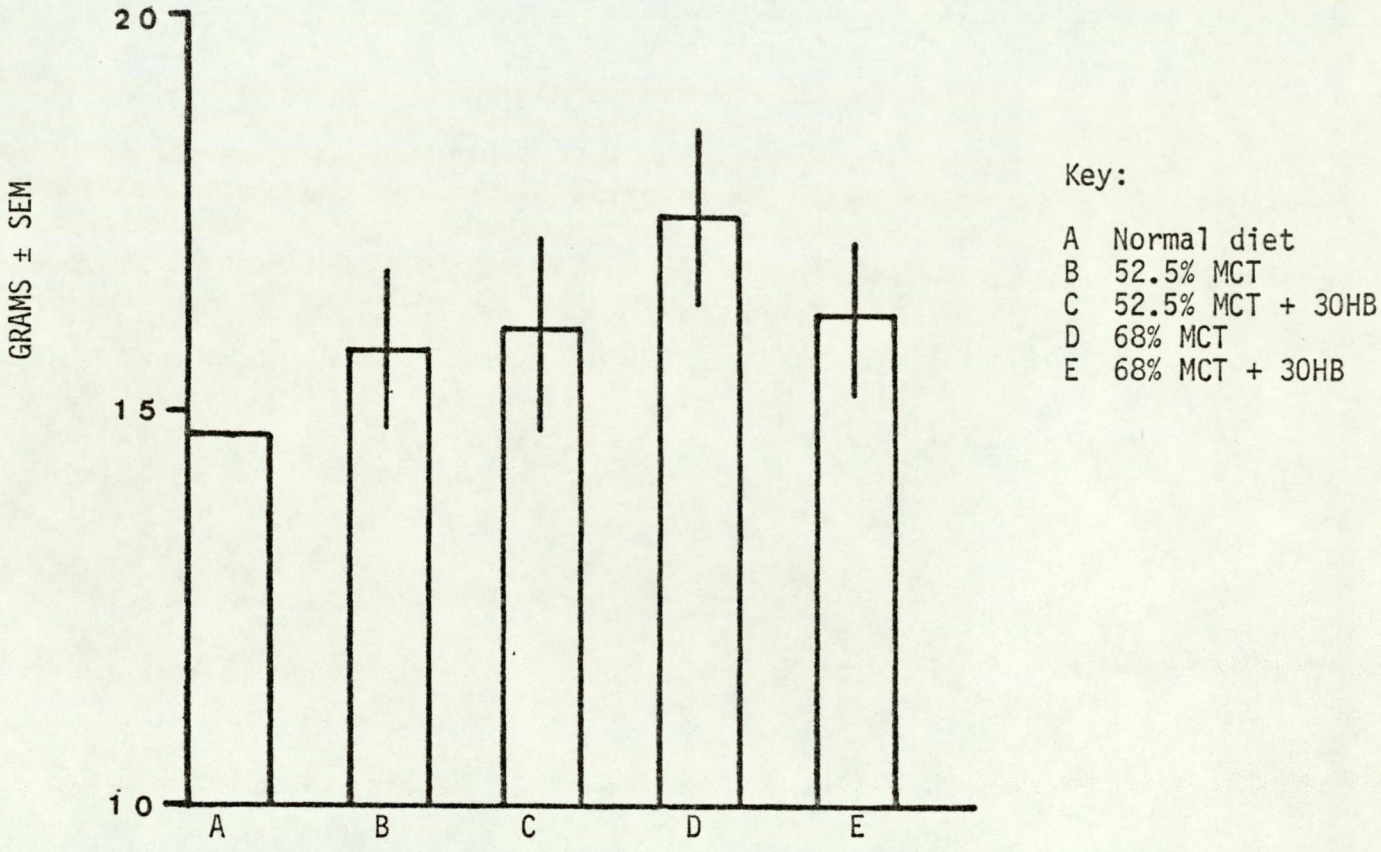


Figure D.3.4 THE EFFECT OF DIETARY MODIFICATION ON TUMOUR WEIGHT OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (EXPRESSED AS TUMOUR WT/20 gm MOUSE)

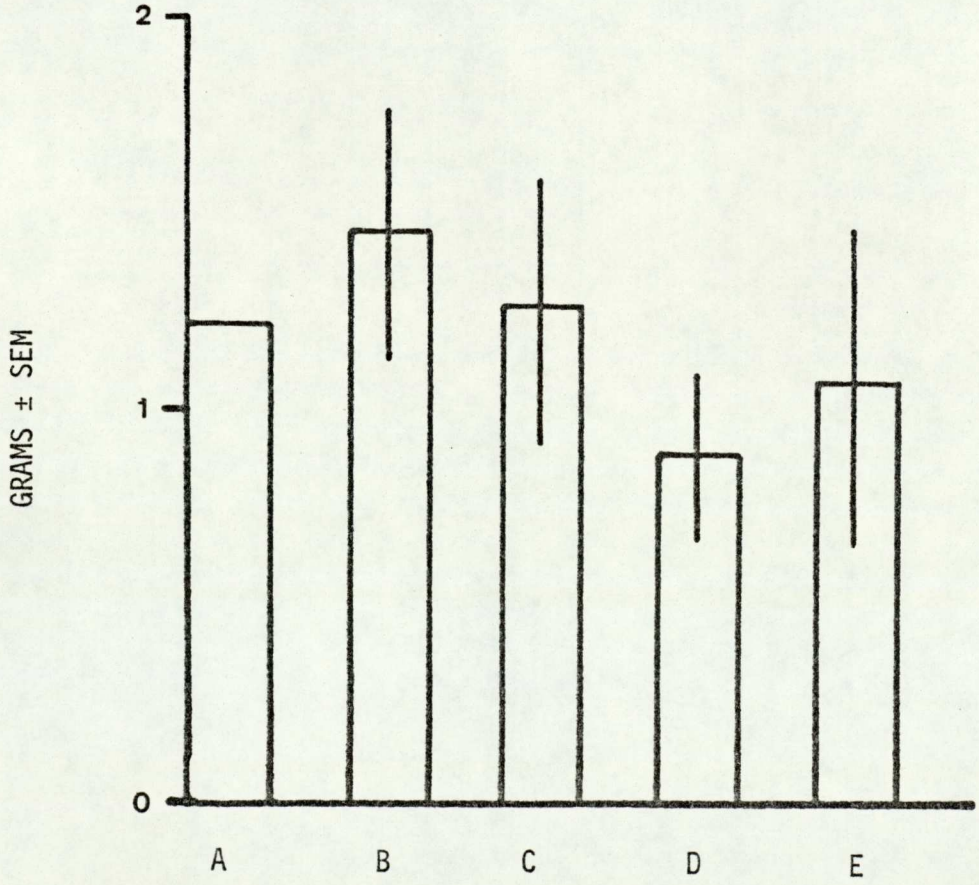


Figure D.3.5 THE EFFECT OF DIETARY MODIFICATION ON THE CARCASS WEIGHT OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (LOWER STARTING BODY WEIGHTS)

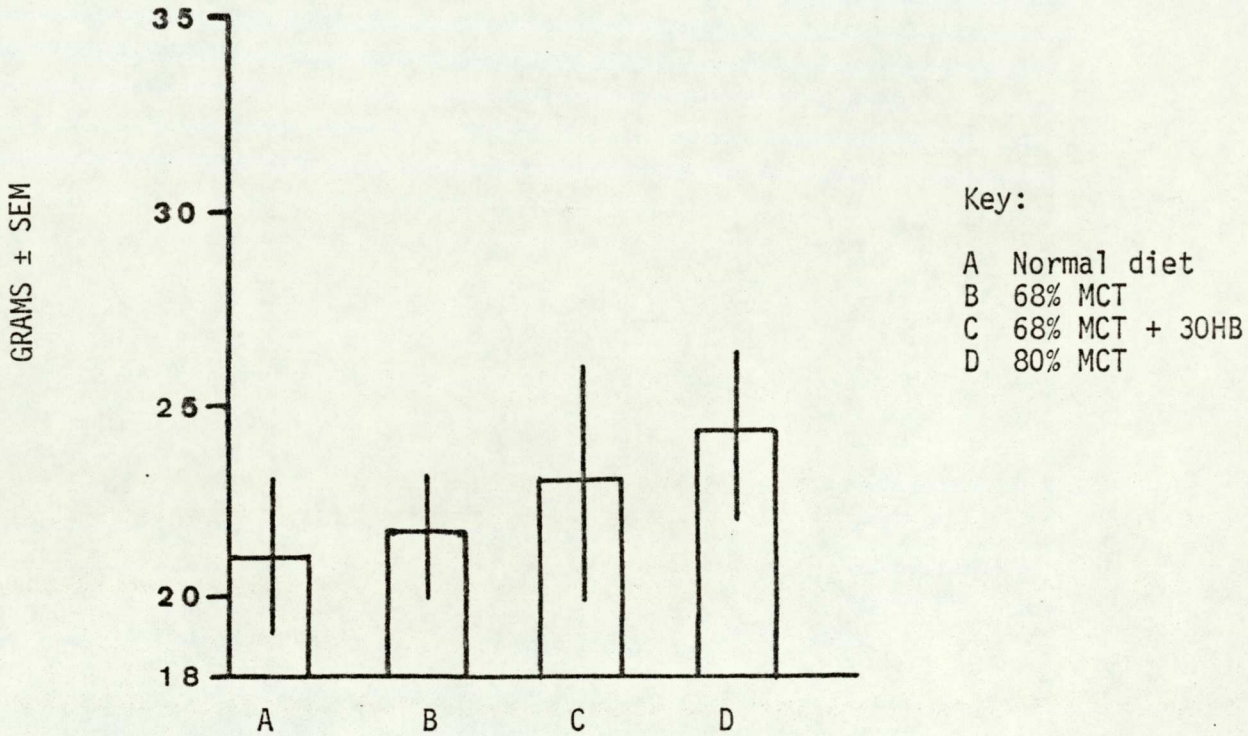


Figure D.3.6 THE EFFECT OF DIETARY MODIFICATION ON TUMOUR WEIGHT OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (LOWER STARTING BODY WEIGHTS, EXPRESSED AS TUMOUR WEIGHT/20 gm MOUSE)

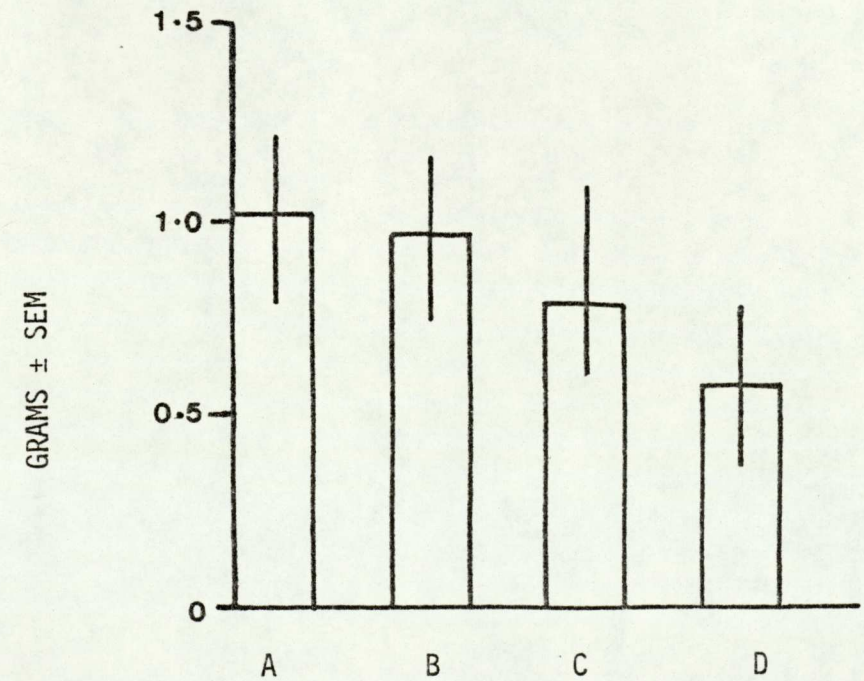


Figure D.3.7 THE EFFECT OF DIETARY MODIFICATION ON THE CARCASS WEIGHT OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

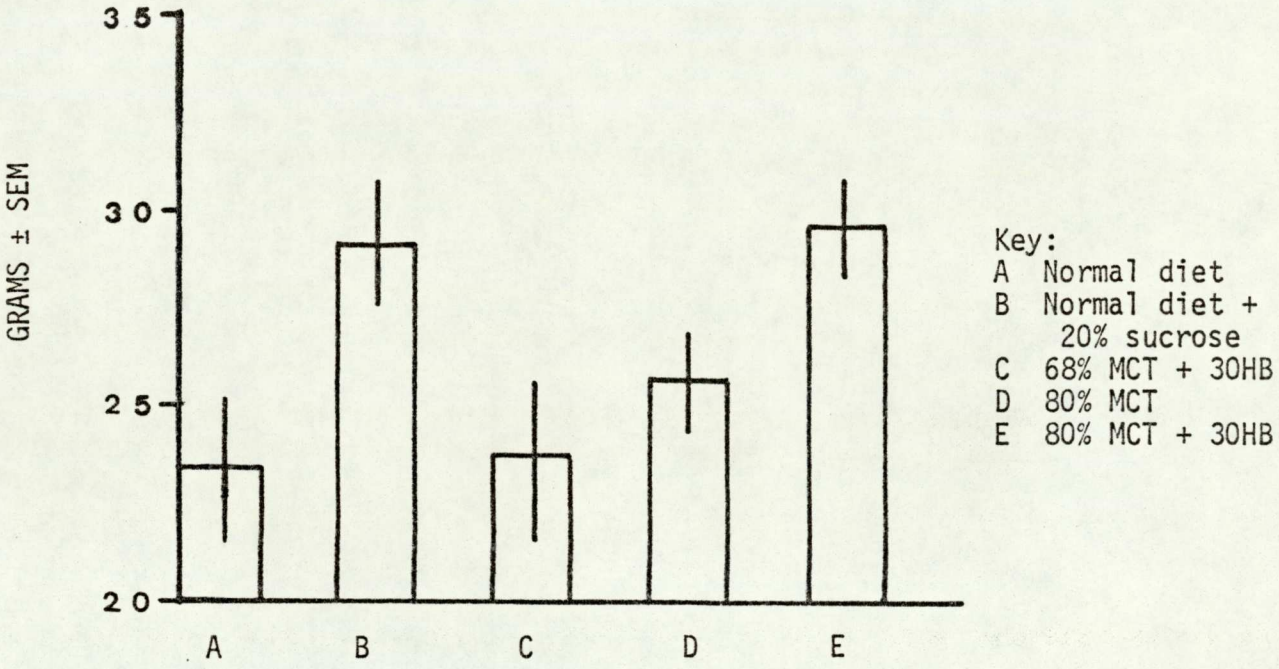


Figure D.3.8 THE EFFECT OF DIETARY MODIFICATION ON TUMOUR WEIGHT OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (EXPRESSED AS TUMOUR WEIGHT/20 gm MOUSE)

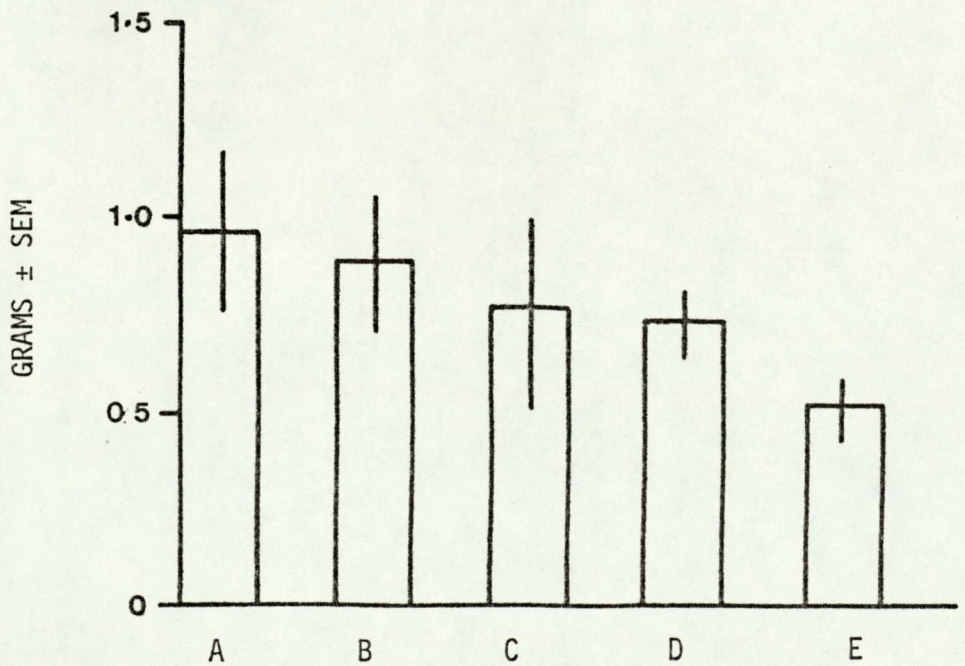


Figure D.3.9 RELATIONSHIP BETWEEN TUMOUR WEIGHT, WEIGHT LOSS AND DIET IN FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA

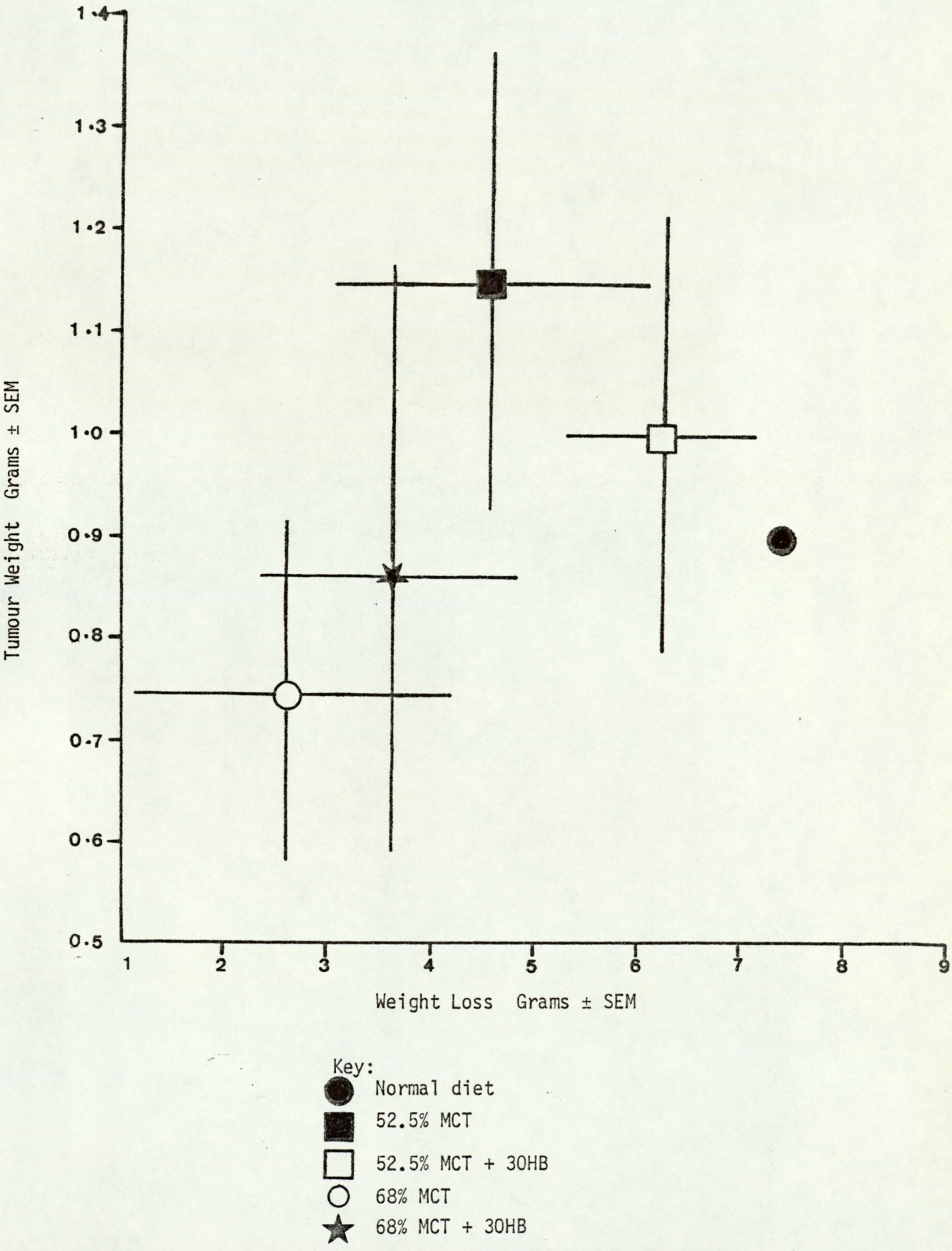
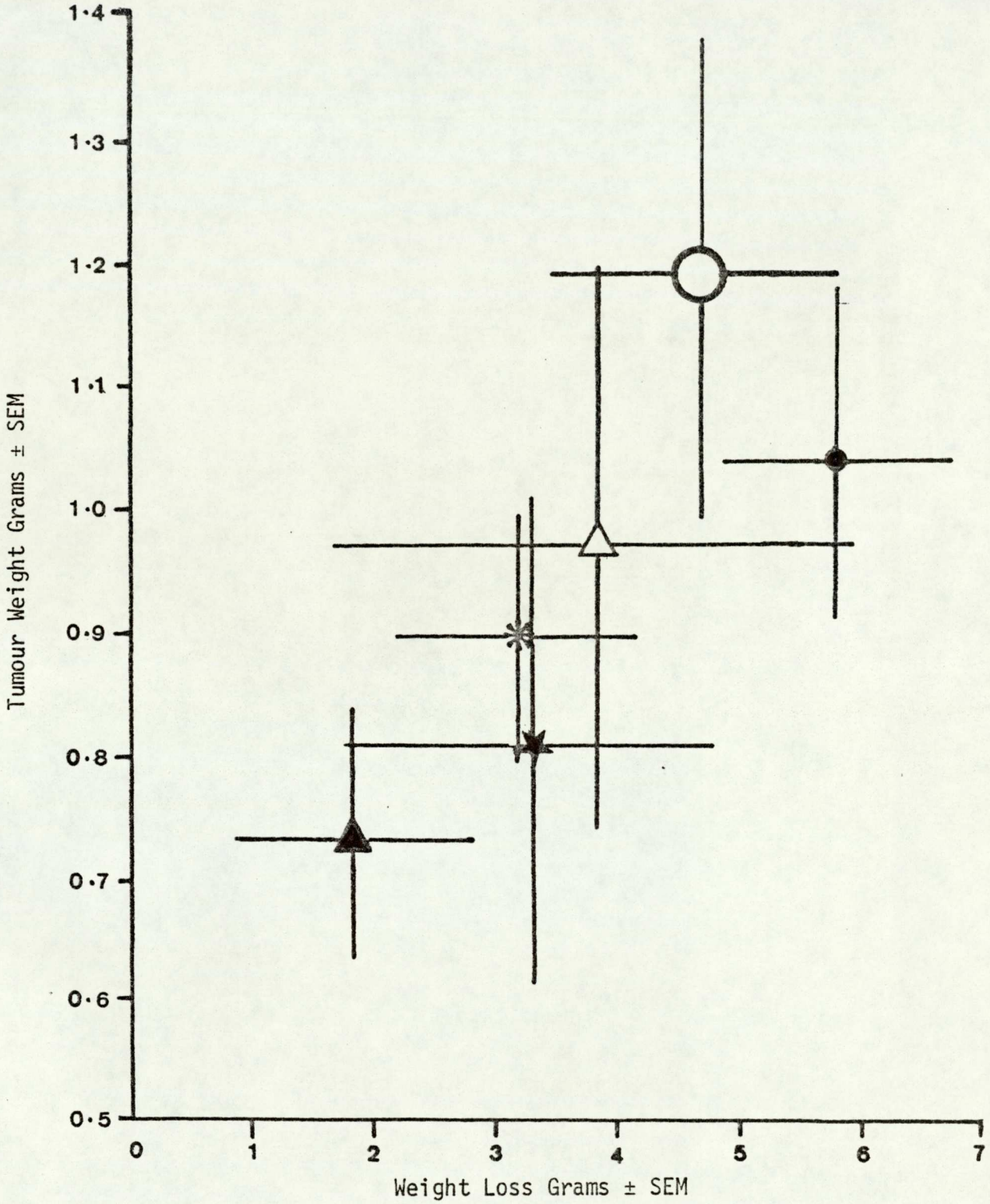


Figure D.3.10 RELATIONSHIP BETWEEN TUMOUR WEIGHT, WEIGHT LOSS AND DIET IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA



Key:

- Normal diet
- Normal diet + 20% sucrose
- 68% MCT
- 68% MCT + 3OHB
- 80% MCT
- 80% MCT + 3OHB

diet supplemented with 3-hydroxybutyrate arginine salt in the studies using mice with the higher starting weights.

The largest average tumour size was seen in the group of animals fed the 52.5% diet in the study using female mice. In the studies using male mice, the largest tumours were seen in the group fed the normal diet. The smallest tumours were seen in the group fed on the 68% MCT diet for female mice, and in the groups fed on the 80% MCT diet, and 80% MCT diet supplemented with 3-hydroxybutyrate arginine salt for the lower starting weight male mice and higher starting weight male mice respectively. Increasing the proportion of triglyceride in the diet and supplementation with 3-hydroxybutyrate arginine salt resulted in a significant reduction ($p < 0.05$) in tumour size in the studies using male mice when tumour weights from these groups were compared with those from mice fed the normal diet. The relationship between tumour weight, weight loss and diet is shown in Figure D.3.9 for female mice, and in Figure D.3.10 for male mice. These results have shown that increasing the amount of fat in the diet leads to a reduction in tumour size which is also associated with a reduction in weight loss.

D.3.3 **Body composition**

The effect of dietary modification on body composition in terms of dry carcass weight, percentage water content and total carcass fat content are shown in Table D.3.9 for female mice and Tables D.3.10 and D.3.11 for male mice and displayed in histogram form in Figures D.3.11 - D.3.19. Mice bearing the MAC 16 tumour show a significant ($p < 0.05$) depression of carcass dry weight when compared

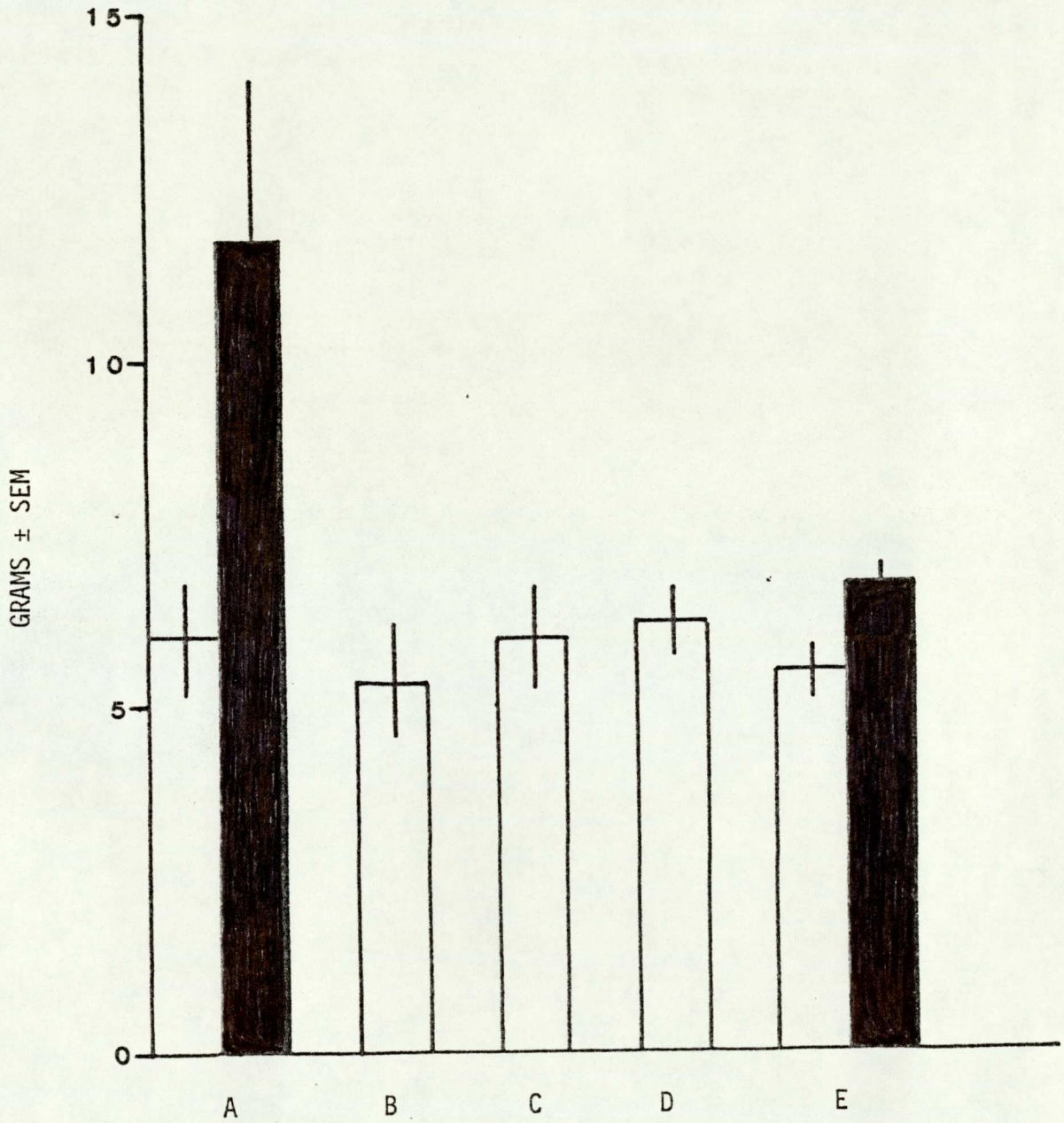
Table D.3.9 THE EFFECT OF DIETARY MODIFICATION ON BODY COMPOSITION OF FEMALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	CARCASS WEIGHT (GRAMS) ± SEM		% WATER CONTENT	TOTAL CARCASS FAT
A	WET WEIGHT	DRY WEIGHT	± SEM	(GRAMS) ± SEM
Normal diet	14.7 ± 1.6	6.0 ± 0.8 [b]	64.3 ± 1.4	0.68 ± 0.4 [b]
52.5% MCT	15.7 ± 1.1	5.3 ± 0.7 [b]	67.8 ± 1.7 [b]	0.50 ± 0.3 [a]
52.5% MCT + 3-hydroxybutyrate	16.1 ± 1.0	6.0 ± 0.7 [b]	63.9 ± 2.4 [b]	0.77 ± 0.3 [a]
68% MCT	17.4 ± 1.1	6.2 ± 0.4 [b]	64.9 ± 0.3 [b]	0.65 ± 0.1 [a]
68% MCT + 3-hydroxybutyrate	16.3 ± 1.0	5.5 ± 0.4	66.2 ± 1.1 [b]	0.58 ± 0.2 [b]
B				
Normal diet	24.1 ± 2.4	11.7 ± 2.4	52.7 ± 5.7	2.62 ± 0.6
68% MCT + 3-hydroxybutyrate	20.1 ± 0.4	6.8 ± 0.1	65.8 ± 1.3	0.96 ± 0.1 [b]

[a] $p < 0.01$ from non tumour-bearing group of mice fed normal diet

[b] $p < 0.05$ from non tumour-bearing group of mice fed normal diet

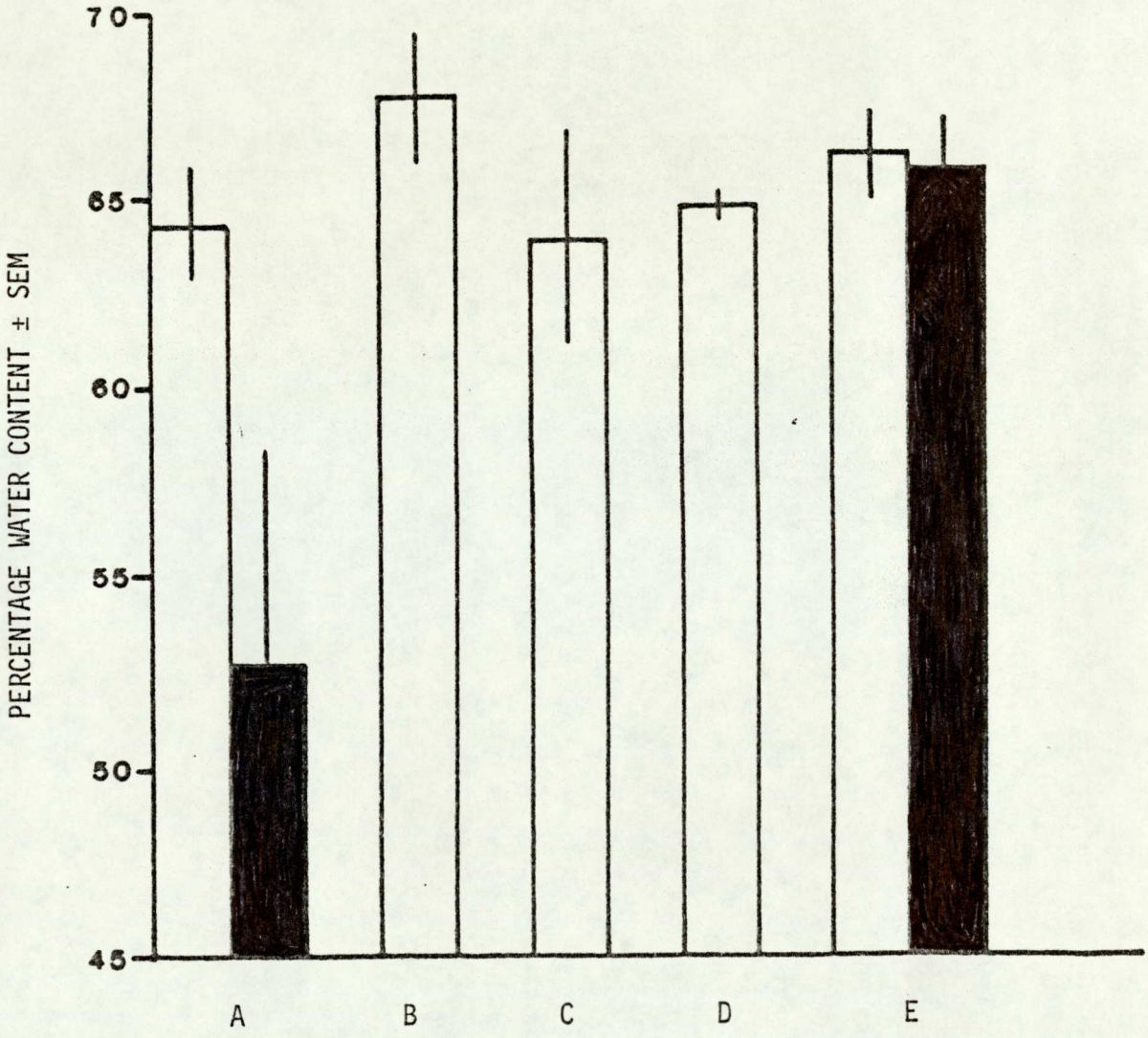
Figure D.3.11 THE EFFECT OF DIETARY MODIFICATION ON THE DRY CARCASS WEIGHTS OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B 52.5% MCT
C 52.5% MCT + 3OHB
D 68% MCT
E 68% MCT + 3OHB

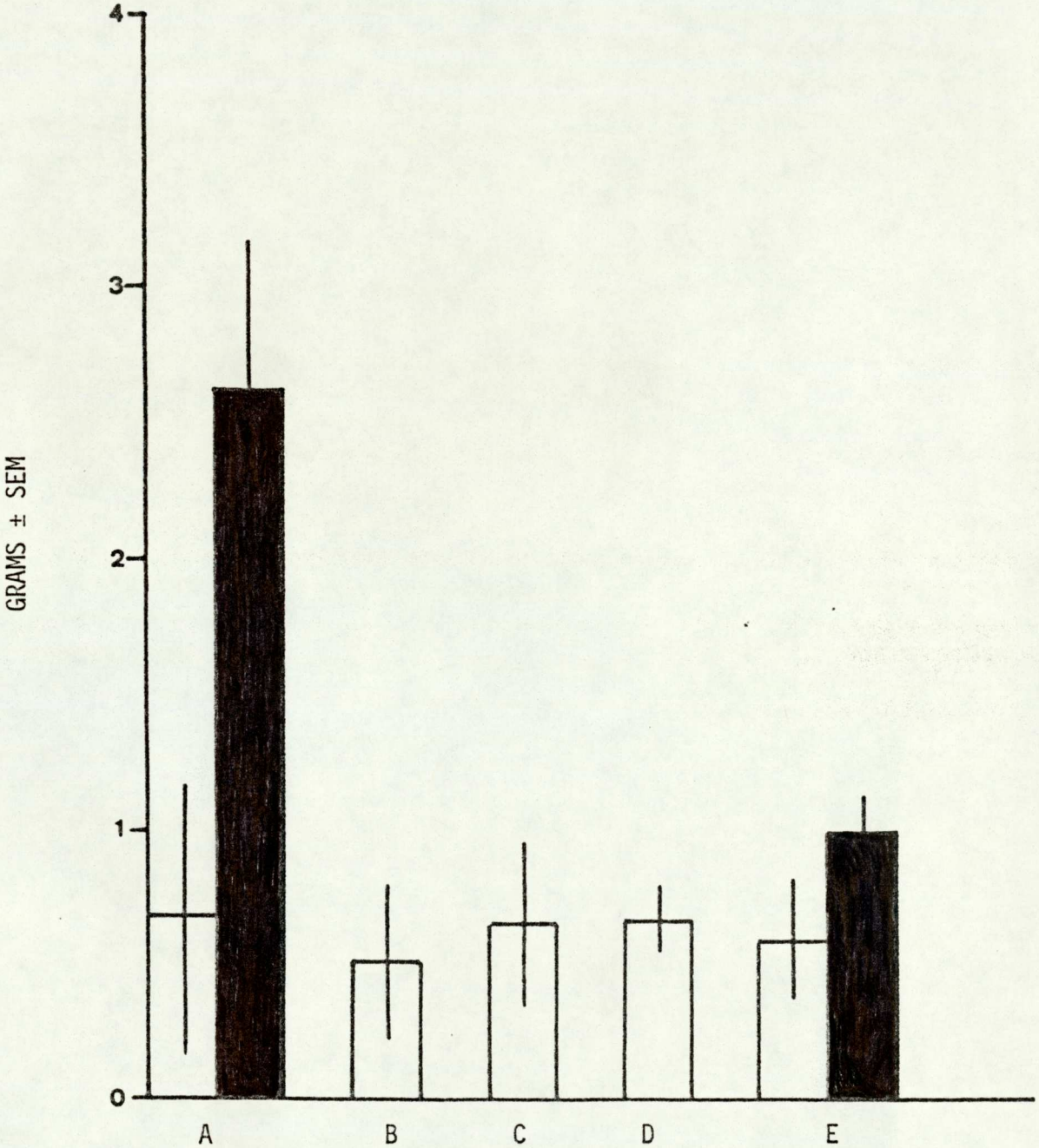
Figure D.3.12 THE EFFECT OF DIETARY MODIFICATION ON THE PERCENTAGE WATER CONTENT OF CARCASSES OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B 52.5% MCT
C 52.5% MCT + 3OHB
D 68% MCT
E 68% MCT + 3OHB

Figure D.3.13 THE EFFECT OF DIETARY MODIFICATION ON THE TOTAL CARCASS FAT CONTENT OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B 52.5% MCT
C 52.5% MCT + 3OHB
D 68% MCT
E 68% MCT + 3OHB

Table D.3.10 THE EFFECT OF DIETARY MODIFICATION ON BODY COMPOSITION OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B). (LOWER STARTING BODY WEIGHTS)

DIETARY TREATMENT	CARCASS WEIGHT (GRAMS) ± SEM		% WATER CONTENT	TOTAL CARCASS FAT
A	WET WEIGHT	DRY WEIGHT	± SEM	(GRAMS) ± SEM
Normal diet	21.1 ± 1.9 [a]	7.1 ± 0.9 [b]	66.6 ± 1.3 [b]	0.41 ± 0.1 [b]
68% MCT	21.7 ± 1.9	6.9 ± 0.6	68.2 ± 0.8	0.48 ± 0.1 [c]
68% MCT + 3-hydroxybutyrate	23.1 ± 3.0	7.5 ± 1.1	67.7 ± 1.0	0.36 ± 0.1 [c]
80% MCT	24.3 ± 2.3 [a]	7.5 ± 0.8	69.1 ± 0.4	0.43 ± 0.1 [c]
B				
Normal diet	30.5 ± 1.8	12.3 ± 0.9	59.8 ± 3.0	1.62 ± 0.3
80% MCT	30.8 ± 1.6	8.8 ± 0.4	71.3 ± 0.4	0.62 ± 0.1

[a] $p < 0.001$ from corresponding non tumour-bearing group of mice

[b] $p < 0.05$ from corresponding non tumour-bearing group of mice

[c] $p < 0.05$ from non tumour-bearing group of mice fed normal diet

Figure D.3.14 THE EFFECT OF DIETARY MODIFICATION ON THE DRY CARCASS WEIGHTS OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES). LOWER STARTING BODY WEIGHTS.

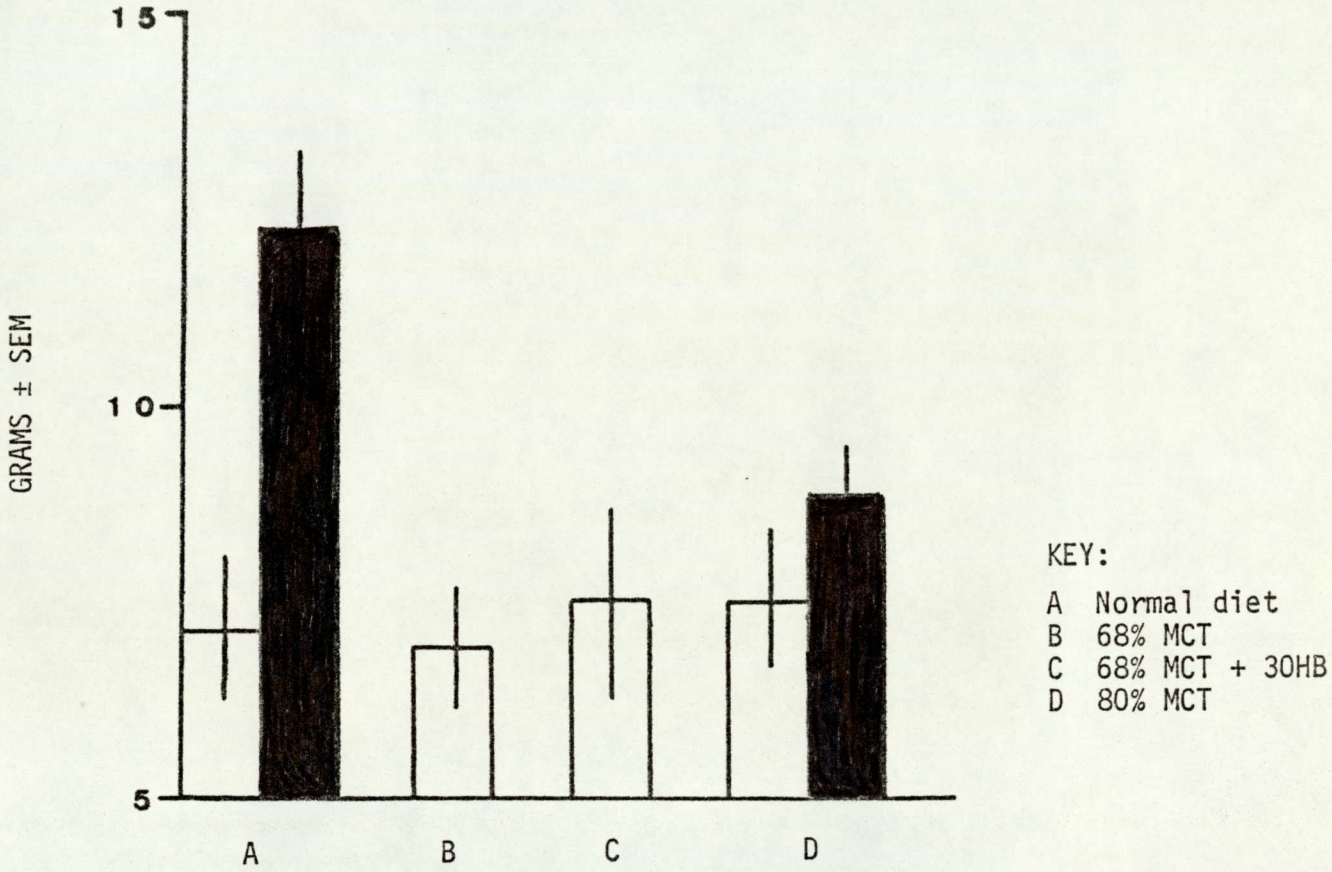


Figure D.3.15 THE EFFECT OF DIETARY MODIFICATION ON THE PERCENTAGE WATER CONTENT OF CARCASSES OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES). LOWER STARTING BODY WEIGHTS.

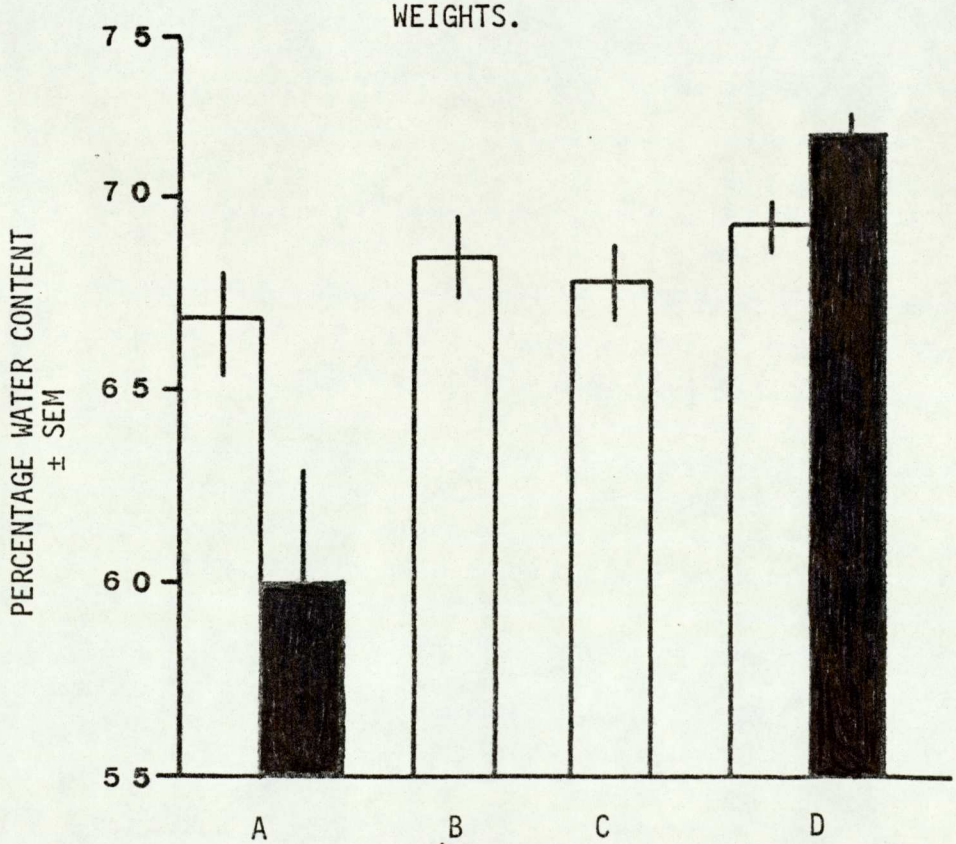


Figure D.3.16 THE EFFECT OF DIETARY MODIFICATION ON THE TOTAL CARCASS FAT CONTENT OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES). LOWER STARTING BODY WEIGHTS.

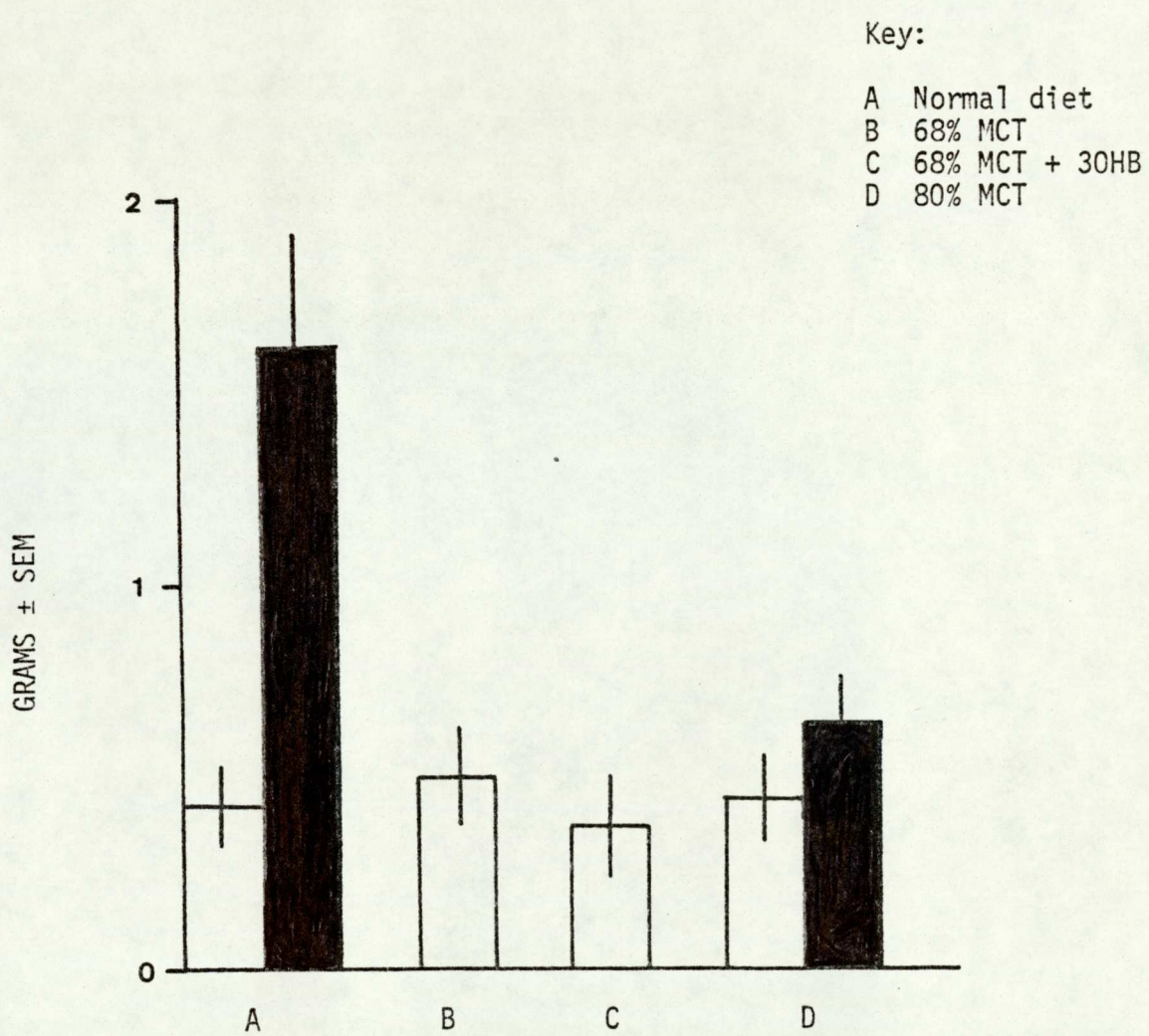


Table D.3.11 THE EFFECT OF DIETARY MODIFICATION ON BODY COMPOSITION OF MALE NMRI MICE BEARING THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	CARCASS WEIGHT (GRAMS) ± SEM		% WATER CONTENT	TOTAL CARCASS FAT
A	WET WEIGHT	DRY WEIGHT	± SEM	(GRAMS) ± SEM
Normal diet	23.4 ± 1.8 [a]	7.4 ± 0.5 [b,d]	66.9 ± 0.8	0.32 ± 0.08 [a]
Normal diet + 20% sucrose	28.1 ± 1.4 [e]	8.9 ± 0.4 [b,f]	69.7 ± 0.3 [a,f,e]	0.77 ± 0.13 [a,c,f]
68% MCT + 3-hydroxybutyrate	23.8 ± 2.1 [b,d,f]	8.1 ± 0.6	68.0 ± 0.6 [b]	0.66 ± 0.13 [c,f]
80% MCT	25.7 ± 1.1 [b,d]	7.9 ± 0.3 [d]	69.3 ± 0.1 [b,e]	0.58 ± 0.07 [c]
80% MCT + 3-hydroxybutyrate	29.7 ± 1.2 [f]	8.7 ± 0.3 [a,f]	71.6 ± 0.6 [a,d,e]	0.53 ± 0.07 [b,c,f]
B				
Normal diet	29.6 ± 0.4	9.5 ± 0.5	66.3 ± 1.5	1.92 ± 0.15
Normal diet + 20% sucrose	32.3 ± 0.9 [e]	10.9 ± 0.3 [e]	66.3 ± 0.7	1.88 ± 0.18 [e]
68% MCT + 3-hydroxybutyrate	33.5 ± 0.3 [f]	9.7 ± 0.7 [f]	71.0 ± 0.7 [f]	0.38 ± 0.18 [c]
80% MCT	30.8 ± 1.2 [f]	8.8 ± 0.4	71.3 ± 0.4 [e]	0.62 ± 0.08 [c,f]
80% MCT + 3-hydroxybutyrate	32.7 ± 1.4 [f]	11.2 ± 0.7 [e]	65.7 ± 1.2	1.00 ± 0.26 [d,e]

Table D.3.11 continued

[a] $p < 0.001$ from corresponding non tumour-bearing group of mice

[b] $p < 0.05$ from corresponding non tumour-bearing group of mice

[c] $p < 0.001$ from non tumour-bearing group of mice fed normal diet

[d] $p < 0.05$ from non tumour-bearing group of mice fed normal diet

[e] $p < 0.002$ from tumour-bearing group of mice fed normal diet

[f] p 0.05 from tumour-bearing group of mice fed normal diet

Figure D.3.17 THE EFFECT OF DIETARY MODIFICATION ON THE DRY CARCASS WEIGHTS OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)

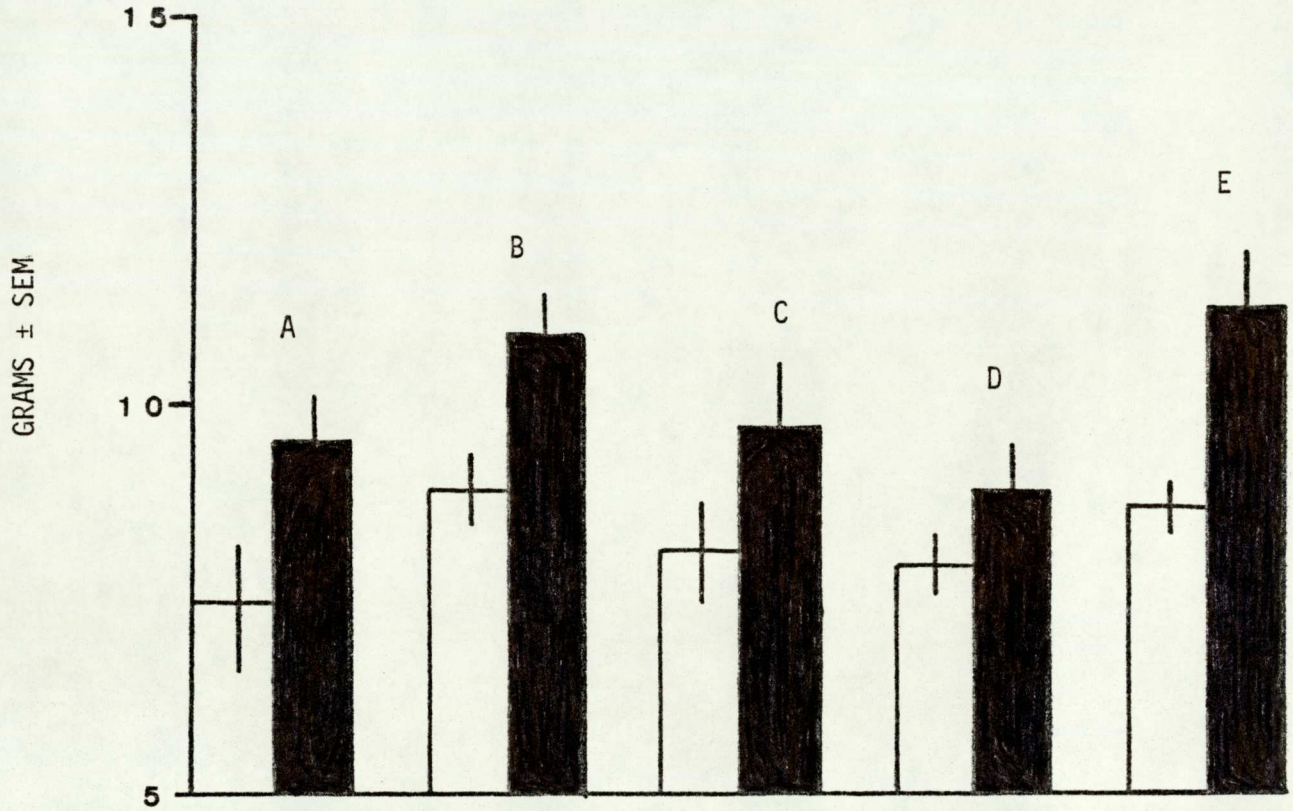
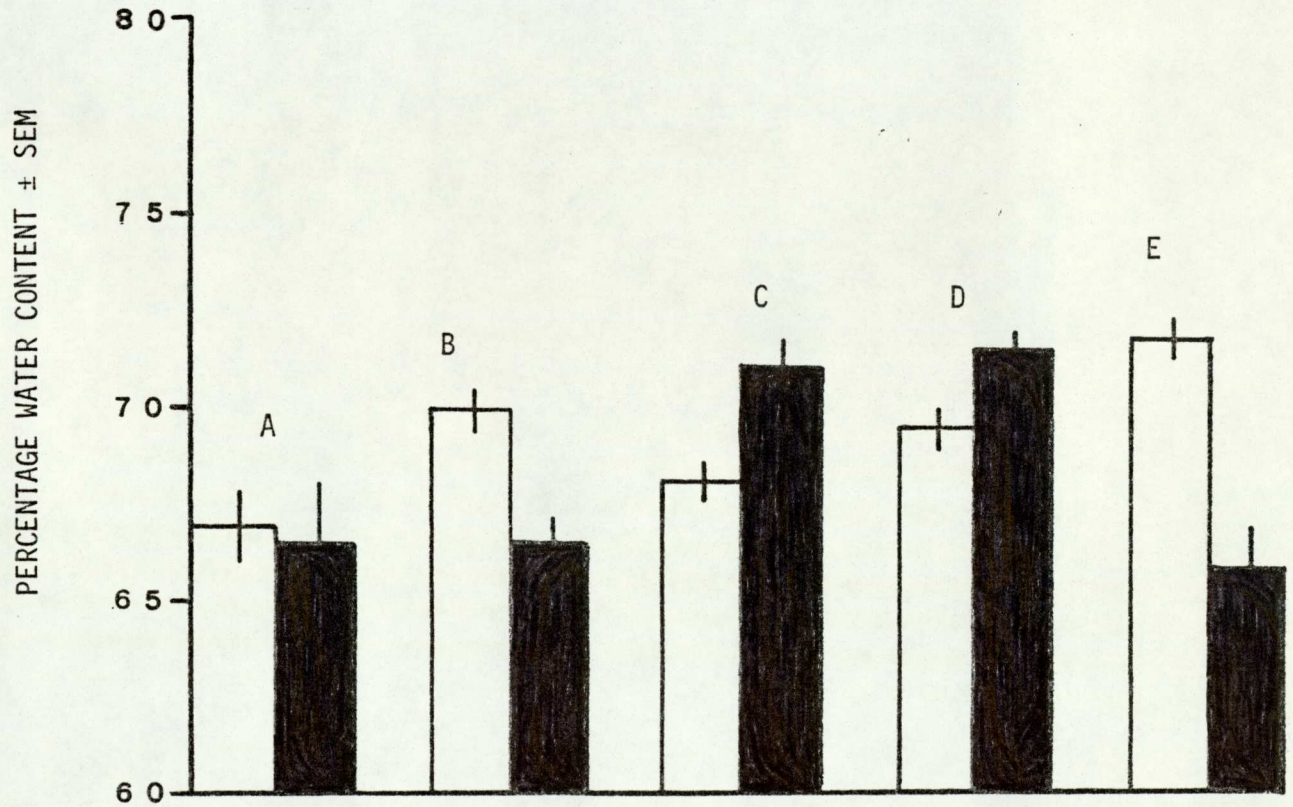
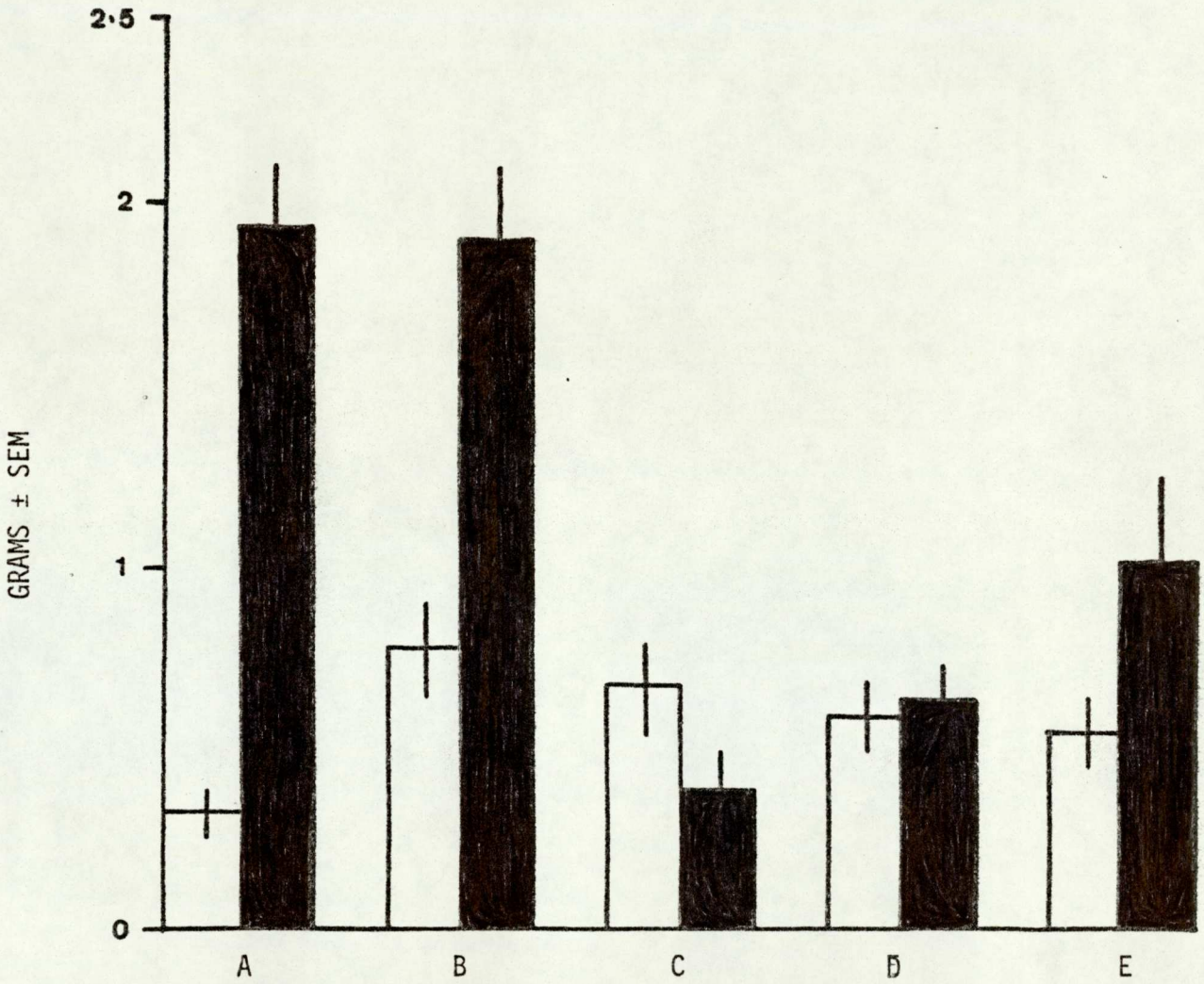


Figure D.3.18 THE EFFECT OF DIETARY MODIFICATION ON THE PERCENTAGE WATER CONTENT OF CARCASSES OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key: A Normal diet
B Normal diet + 20% sucrose
C 68% MCT + 3OHB
D 80% MCT
E 80% MCT + 3OHB

Figure D.3.19 THE EFFECT OF DIETARY MODIFICATION ON THE TOTAL CARCASS FAT CONTENT OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B Normal diet + 20% sucrose
C 68% MCT + 3OHB
D 80% MCT
E 80% MCT + 3OHB

with the control groups of non tumour-bearing mice. The total fat content of the carcasses of tumour-bearing mice were significantly decreased ($p < 0.05$) compared to non tumour-bearing mice fed normal diets. The depletion of body fat is prevented to some extent by increasing the contribution of energy derived from triglycerides, total carcass fat being significantly higher ($p < 0.05$) in all groups of tumour-bearing male NMRI mice fed high MCT diets both with and without 3-hydroxybutyrate arginine salt. Dietary modification also had a similar effect on preserving the carcass fat content of female tumour-bearing mice. Increasing the fat content of the diet also decreased the carcass fat content of non tumour-bearing mice, probably due to insufficient carbohydrate to supply oxaloacetate for citrate formation. Depletion of carcass fat was also reduced in tumour-bearing mice fed normal diets supplemented with 20% sucrose, total carcass fat content being significantly higher ($p < 0.05$) than that of the tumour-bearing mice fed on normal diet only.

The percentage water content of carcasses of tumour-bearing mice were not altered by dietary modification. Thus, the decrease in weight reduction which occurred in tumour-bearing mice fed diets with increasing proportions of energy derived from MCT arises mainly from an increase in non-fat carcass mass.

Tables D.3.12 and D.3.13 show the effect of dietary modification on the thigh and gastrocnemius muscle weights in female and male mice respectively. In the study using female mice, thigh muscle weights for all the tumour-bearing groups of mice were significantly lower ($p < 0.05$) than those of the non tumour-bearing group fed on normal pelleted food. Gastrocnemius muscle weights were also lower in all the groups of tumour-bearing animals compared to the non tumour-bearing control groups fed on the normal diet and the high MCT diets

Table D.3.12 THE EFFECT OF DIETARY MODIFICATION ON THIGH AND GASTROCNEMIUS MUSCLE WEIGHTS OF FEMALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	THIGH MUSCLE		%	GASTROCNEMIUS MUSCLE		%
	WET WEIGHT mg ± SEM	DRY WEIGHT mg ± SEM	CARCASS WEIGHT ± SEM	WET WEIGHT mg ± SEM	DRY WEIGHT mg ± SEM	CARCASS WEIGHT ± SEM
A						
Normal diet	102±17.0 [a]	26.5±4.2 [a]	0.60±0.06	124±16.3	33.0±5.2	0.73±0.05
52.5% MCT	114±8.7 [a]	29.7±6.6	0.71±0.01	106±21.3	24.0±5.5	0.66±0.13
52.5% MCT + 3-hydroxybutyrate	121±7.1 [a]	29.8±4.2	0.73±0.06	116±16.3 [a]	32.7±1.9 [a]	0.69±0.06
68% MCT	123±13.5[a]	31.1±3.6	0.69±0.05	116±25.6	30.1±6.3	0.65±0.13
68% MCT + 3-hydroxybutyrate	117±19.7 [a]	28.6±5.8	0.69±0.10	132±21.3 [a]	34.5±5.4 [a]	0.78±0.12
B						
Normal diet	169±14.7	39.7±4.8	0.72±0.09	150±7.5	40.8±2.6	0.64±0.06
68% MCT + 3-hydroxybutyrate	129±10.6 [a]	31.0±2.3	0.64±0.05	146±11.2	35.5±1.9	0.73±0.07

[a] $p < 0.05$ from non tumour-bearing group of mice fed normal diet

Table D.3.13 THE EFFECT OF DIETARY MODIFICATION ON THIGH AND GASTROCNEMIUS MUSCLE WEIGHTS OF MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	THIGH MUSCLE			GASTROCNEMIUS MUSCLE		
	WET WEIGHT mg ± SEM	DRY WEIGHT mg ± SEM	% CARCASS WEIGHT ± SEM	WET WEIGHT mg ± SEM	DRY WEIGHT mg ± SEM	% CARCASS WEIGHT ± SEM
A						
Normal diet	174±4.7 [b]	44.5±0.1 [b]	0.84±0.08	197±30.6	50.4±7.9	0.93±0.13
68% MCT	163±18.2 [b]	33.6±3.7 [b,c]	0.76±0.07	156±20.4 [a,b]	38.3±5.8 [a]	0.71±0.04
68% MCT + 3-hydroxybutyrate	145±19.7 [b]	34.3±5.1 [b]	0.63±0.03 [c]	198±24.4	47.6±6.6	0.85±0.11
80% MCT	175±17.5 [b]	43.4±5.8	0.72±0.02	208±21.1	49.3±6.6	0.86±0.04
B						
Normal diet	198±10.7	50.0±6.6	0.65±0.06	26.2±22.1	66.8±8.4	0.86±0.07
80% MCT	220±15.5	55.9±4.9	0.72±0.05	217±19.2	52.3±6.4	0.71±0.05

[a] $p < 0.05$ from non tumour-bearing group of mice fed normal diet

[b] $p < 0.05$ from non tumour-bearing group of mice fed 80% MCT diet

[c] $p < 0.05$ from tumour-bearing group of mice fed normal diet

supplemented with 3-hydroxybutyrate arginine salt. In the study using male mice, thigh muscle weights and gastrocnemius muscle weights in all the tumour-bearing groups were lower than those from the non tumour-bearing groups of mice, although these differences were not significant. The wet weights of the muscles were expressed as a percentage of the carcass weight, and these show that there was little difference between any of the groups; loss of muscle in terms of weight being proportional to the weight of the carcass.

D.3.4 **Food consumption**

Tables D.3.14 and D.3.15 show the total amount of calories consumed per mouse for each of the diet treatment groups. Female mice (Table D.3.14) consumed less food and therefore fewer calories than male mice (Table D.3.15), probably because they were of much lower body weight. Tumour-bearing female mice on the normal pelleted diet consumed fewer calories than the corresponding non tumour-bearing group overall. Mice on the high MCT diets in both studies consumed more food and therefore more calories than mice on the normal pelleted diets. Food consumption and therefore the total number of calories consumed per mouse were similar in tumour-bearing and non tumour-bearing groups of mice fed the same high MCT diets. There was no reduction in food consumption over the duration of the studies for any of the groups to account for the weight loss in the tumour-bearing animals.

D.3.5 **Levels of activity of 3-oxo acid-CoA transferase**

Levels of activity of 3-oxo acid-CoA transferase measured in

Table D.3.14 TOTAL CALORIE CONSUMPTION OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	TOTAL NUMBER OF CALORIES CONSUMED PER MOUSE Kcal
A	
Normal diet	160.9
52.5% MCT	276.8
52.5% MCT + 3-hydroxybutyrate	335.7
68% MCT	296.8
68% MCT + 3-hydroxybutyrate	348.8
B	
Normal diet	209.9
68% MCT + 3-hydroxybutyrate	228.0

Table D.3.15 TOTAL CALORIE CONSUMPTION OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	TOTAL NUMBER OF CALORIES CONSUMED PER MOUSE Kcal
A	
Normal diet	201.5
Normal diet + 20% sucrose	224.4
68% MCT	341.9
68% MCT + 3-hydroxybutyrate	340.2
80% MCT	346.9
80% MCT + 3-hydroxybutyrate	330.1
B	
Normal diet	200.8
Normal diet + 20% sucrose	189.2
68% MCT	326.6
68% MCT + 3-hydroxybutyrate	352.3
80% MCT	334.6
80% MCT + 3-hydroxybutyrate	326.9

tumours taken from female NMR I mice are shown in Table D.3.16 and Figure D.3.20. The level of activity of the enzyme was measured in the direction of acetoacetyl-CoA formation only, ie; forward direction, and was highest in the tumours from mice fed on the normal pelleted diet compared to levels of activity found in tumours taken from the groups of mice fed the high MCT diets. The lowest levels of activity were found in the groups of mice fed on the 52.5% MCT diet, and the 68% MCT diet supplemented with 3-hydroxybutyrate arginine salt, and these were significantly lower ($p > 0.02$) than the levels measured in tumours taken from the group fed the normal diet. The levels of activity measured in this study are much lower than those measured previously in the tumours in the enzyme study (Section D.1.2). Dietary modification did not lead to an increase in the levels of activity of the enzyme 3-oxo acid-CoA transferase in the tumours of animals fed the high MCT diets.

D.3.6 **Metabolite studies**

The levels of blood metabolites from NMR I mice fed the various diets are shown in Table D.3.17 for female mice, and in Tables D.3.18 and D.3.19 for male mice. In the study using female mice it is not possible to say whether any differences observed in the levels of metabolites between the different diet treatment groups are significant, since measurements were made on pooled blood samples. The results shown for the male mice are the aggregates of several experiments.

Table D.3.16 THE EFFECT OF DIETARY MODIFICATION ON 3-OXO ACID-CoA TRANSFERASE LEVELS IN THE MAC 16 ADENOCARCINOMA FROM FEMALE NMR I MICE

DIETARY TREATMENT	μmoles/min/mg Protein ± SEM
Normal diet	0.67 ± 0.09
52.5% MCT	0.18 ± 0.04 [a]
52.5% MCT + 3-hydroxybutyrate	0.28 ± 0.05
68% MCT	0.35 ± 0.02
68% MCT + 3-hydroxybutyrate	0.20 ± 0.05 [a]

[a] $p < 0.002$ from group of mice fed on normal diet

Figure D.3.20 THE EFFECT OF DIETARY MODIFICATION ON LEVELS OF ACTIVITY OF 3-OXO ACID-CoA TRANSFERASE IN THE MAC 16 ADENOCARCINOMA FROM FEMALE NMR I MICE

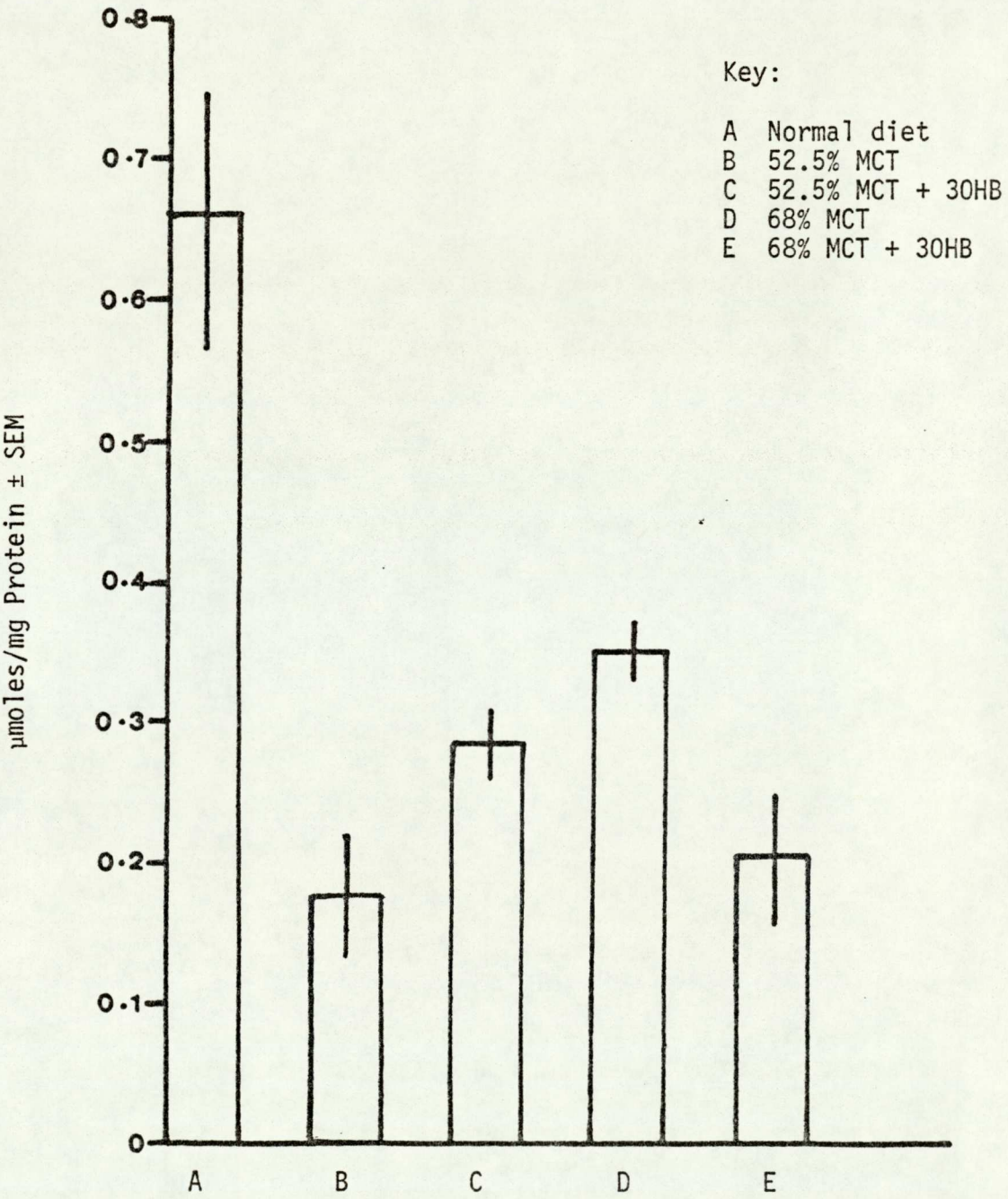


Table D.3.17 THE EFFECT OF DIETARY MODIFICATION ON BLOOD METABOLITE LEVELS IN FEMALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	GLUCOSE mg/100 ml/blood	LACTATE mM	FFA mM	ACETOACETATE mM	3-HYDROXYBUTYRATE mM
A					
Normal diet	110.0	7.6	0.46	0.07	0.15
52.5% MCT	140.3	8.2	0.69	0.09	0.23
52.5% MCT + 3-hydroxybutyrate	118.9	8.0	0.49	0.07	0.24
68% MCT	135.3	8.4	0.58	0.09	0.24
68% MCT + 3-hydroxybutyrate	104.3	6.8	0.63	0.12	0.32
B					
Normal diet	163.9	10.5	0.51	0.07	0.08
68% MCT + 3-hydroxybutyrate	195.0	9.5	0.51	0.06	0.18

Table D.3.18 THE EFFECT OF DIETARY MODIFICATION ON BLOOD LEVELS OF FFA, ACETOACETATE AND 3-HYDROXYBUTYRATE IN MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND NON TUMOUR-BEARING MICE (B)

DIETARY TREATMENT	FFA mM	ACETOACETATE mM	3-HYDROXYBUTYRATE mM
A			
Normal diet	1.01±0.16	0.05±0.02	0.09±0.02
Normal diet + 20% sucrose	1.01±0.19	0.06±0.06	0.08±0.02
68% MCT	0.80±0.15	0.17±0.05 [a,c]	0.34±0.06 [b]
68% MCT + 3-hydroxybutyrate	0.49±0.09 [b]	0.12±0.03 [a,c]	0.28±0.03 [b]
80% MCT	0.77±0.07 [a]	0.31±0.10 [c]	0.34±0.07 [b]
80% MCT + 3-hydroxybutyrate	0.76±0.05 [a]	0.50±0.15 [c]	0.28±0.09 [c]
B			
Normal diet	0.89±0.07	0.05±0.01	0.08±0.01
Normal diet + 20% sucrose	0.74±0.07	0.04±0.04	0.06±0.01
68% MCT	1.28±0.29	0.65±0.19 [d]	0.17±0.07
68% MCT + 3-hydroxybutyrate	0.45±0.13	0.64±0.06 [d]	0.38±10.11
80% MCT	0.39±0.05	0.14±0.05	0.18±0.05
80% MCT + 3-hydroxybutyrate	0.48±0.09	0.47±0.06	0.34±0.04

[a] $p < 0.05$ from corresponding non tumour-bearing group
[b] $p < 0.005$ from tumour-bearing group on normal diet
[c] $p < 0.05$ from tumour-bearing group on normal diet
[d] $p < 0.05$ from non tumour-bearing group on normal diet

Table D.3.19 THE EFFECT OF DIETARY MODIFICATION ON BLOOD LEVELS OF INSULIN, GLUCOSE AND LACTATE IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (A) AND IN NON TUMOUR-BEARING MICE (B)

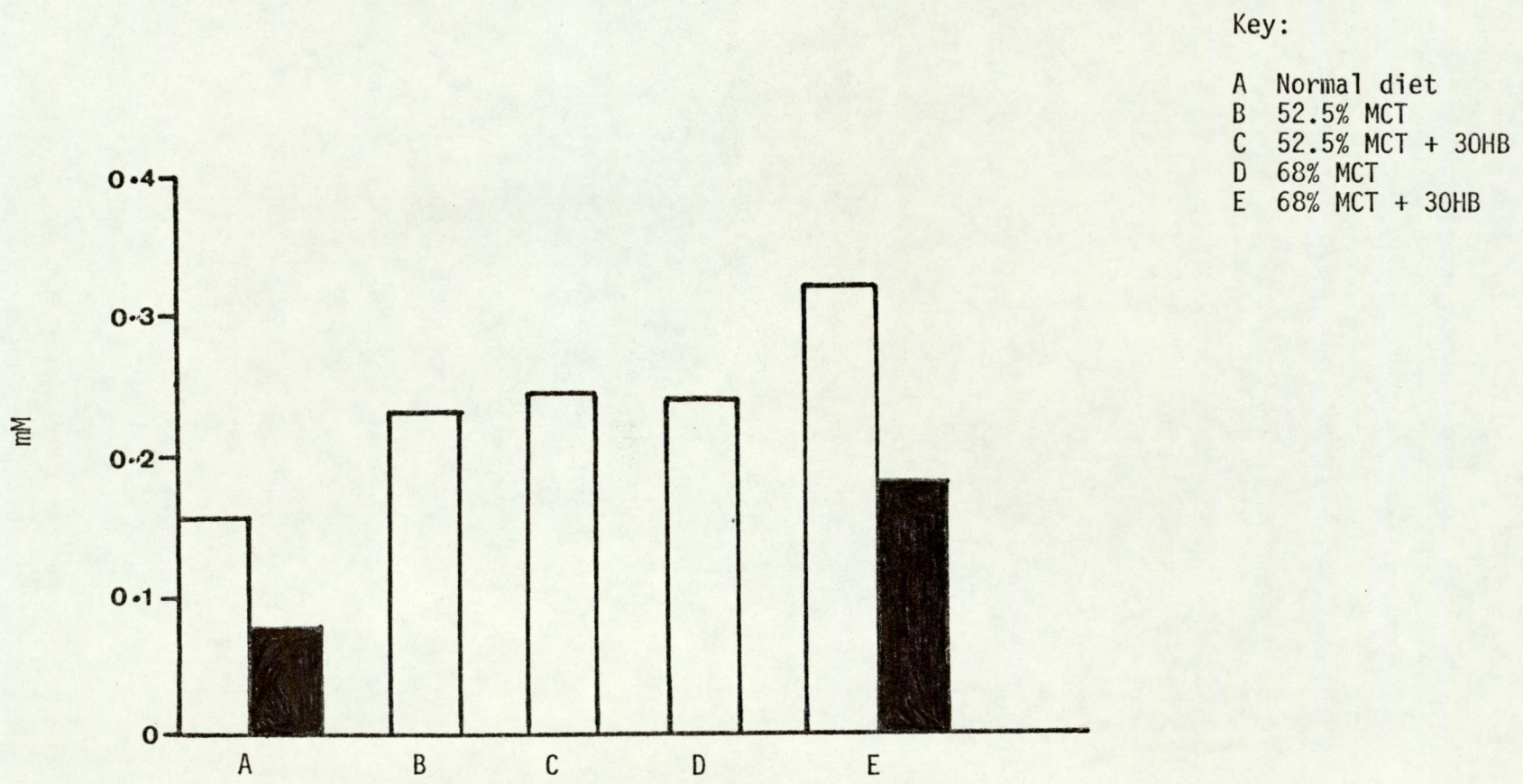
DIETARY TREATMENT	INSULIN ng/ml ± SEM Plasma	GLUCOSE mg/100 ml ± SEM blood	LACTATE mM ± SEM
A			
Normal diet	0.67 ± 0.07 [b,d]	97.5 ± 9.42 [b]	7.3 ± 0.9
Normal diet + 20% sucrose	0.86 ± 0.12 [d,c]	98.3 ± 4.76 [b]	9.8 ± 1.2
68% MCT	1.02 ± 0.39	117.2 ± 20.4	3.3 ± 0.9 [a,c]
68% MCT + 3-hydroxybutyrate	0.94 ± 0.16 [d]	81.1 ± 7.08 [b]	5.6 ± 1.2
80% MCT	0.70 ± 0.10 [c,d]	101.4 ± 8.87	6.5 ± 0.7
80% MCT + 3-hydroxybutyrate	0.54 ± 0.03 [b,d]	112.3 ± 12.4	6.2 ± 0.4
B			
Normal diet	1.66 ± 0.21	137.6 ± 7.98	6.8 ± 0.8
Normal diet + 20% sucrose	2.26 ± 0.48 [e]	131.7 ± 8.3	7.3 ± 0.6
68% MCT	0.72	121.7 ± 6.7	5.6 ± 0.2
68% MCT + 3-hydroxybutyrate	1.07 ± 0.14	127.0 ± 17.6	5.9 ± 1.3
80% MCT	1.20 ± 0.11	129.7 ± 3.0	3.3 ± 0.9
80% MCT + 3-hydroxybutyrate	0.81 ± 0.08	95.7 ± 14.6	6.4 ± 1.2

[a] $p < 0.02$ from tumour-bearing group of mice on normal diet
[b] $p < 0.01$ from corresponding non tumour-bearing group of mice
[c] $p < 0.05$ from corresponding non tumour-bearing group of mice
[d] $p < 0.05$ from non tumour-bearing group of mice on normal diet
[e] $p < 0.05$ from all other groups of mice

D.3.6.1 Levels of 3-hydroxybutyrate and acetoacetate

The levels of these metabolites are displayed in histogram form in Figures D.3.21 and D.3.22 for female mice, and Figures D.3.23 and D.3.24 for male mice. Levels of 3-hydroxybutyrate and acetoacetate in tumour-bearing mice fed the normal diet were not elevated above those of the non tumour-bearing control group, despite the considerable loss of carcass fat. There was a significant increase ($p < 0.05$) in plasma levels of 3-hydroxybutyrate and acetoacetate in both tumour-bearing and non tumour-bearing male mice fed high MCT diets over those mice fed the normal diet. Blood ketone body levels in tumour-bearing groups fed the 80% MCT diet both with and without 3-hydroxybutyrate were similar to those in the corresponding non tumour-bearing groups. In the study using male mice, levels of acetoacetate were significantly lower ($p < 0.05$) in the tumour-bearing groups of mice fed the 68% MCT diet both with and without 3-hydroxybutyrate compared to levels in the corresponding non tumour-bearing groups. Supplementation of the high MCT diets with 3-hydroxybutyrate did not result in a significant elevation of ketone body levels over groups of mice fed corresponding high MCT diets without it. This may be due to a high metabolic activity in mice, and the excretion of substantial amounts of ketone bodies in the urine of mice fed the high MCT diets (tested by Ketostix, results not shown). The 3-hydroxybutyrate:acetoacetate ratio was also altered by increasing the amount of fat in the diet, a reduction occurring in the study using male mice. In the study using female mice the ratio was increased. The rate of ketone body metabolism in peripheral tissues has been shown to correlate directly with prevailing blood concentrations (Bates et al, 1968; Reed et al, 1984), except at high

Figure D.3.21 THE EFFECT OF DIETARY MODIFICATION ON LEVELS OF 3-HYDROXYBUTYRATE IN THE BLOOD OF FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND IN NON TUMOUR-BEARING MICE (BLACK BOXES)



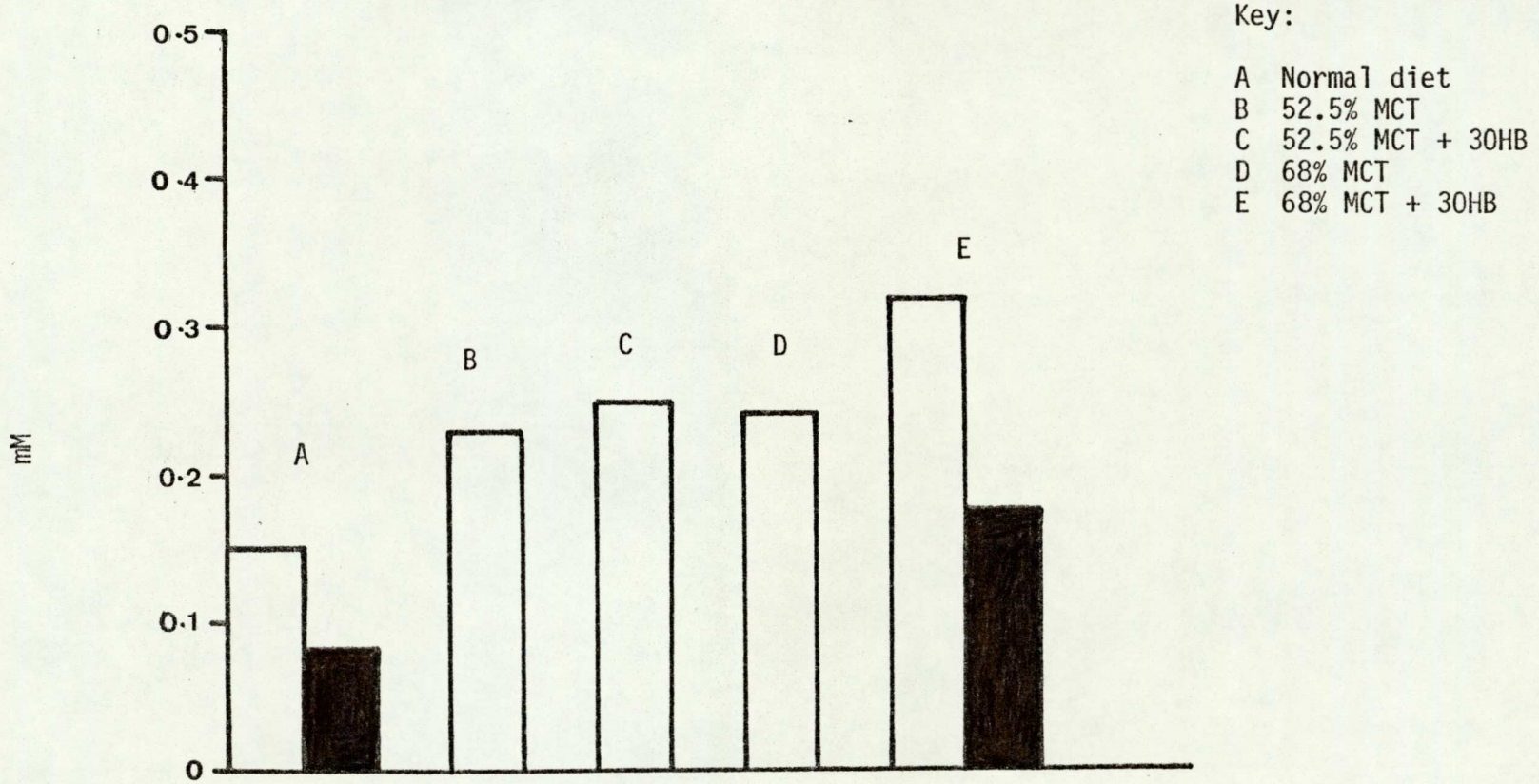
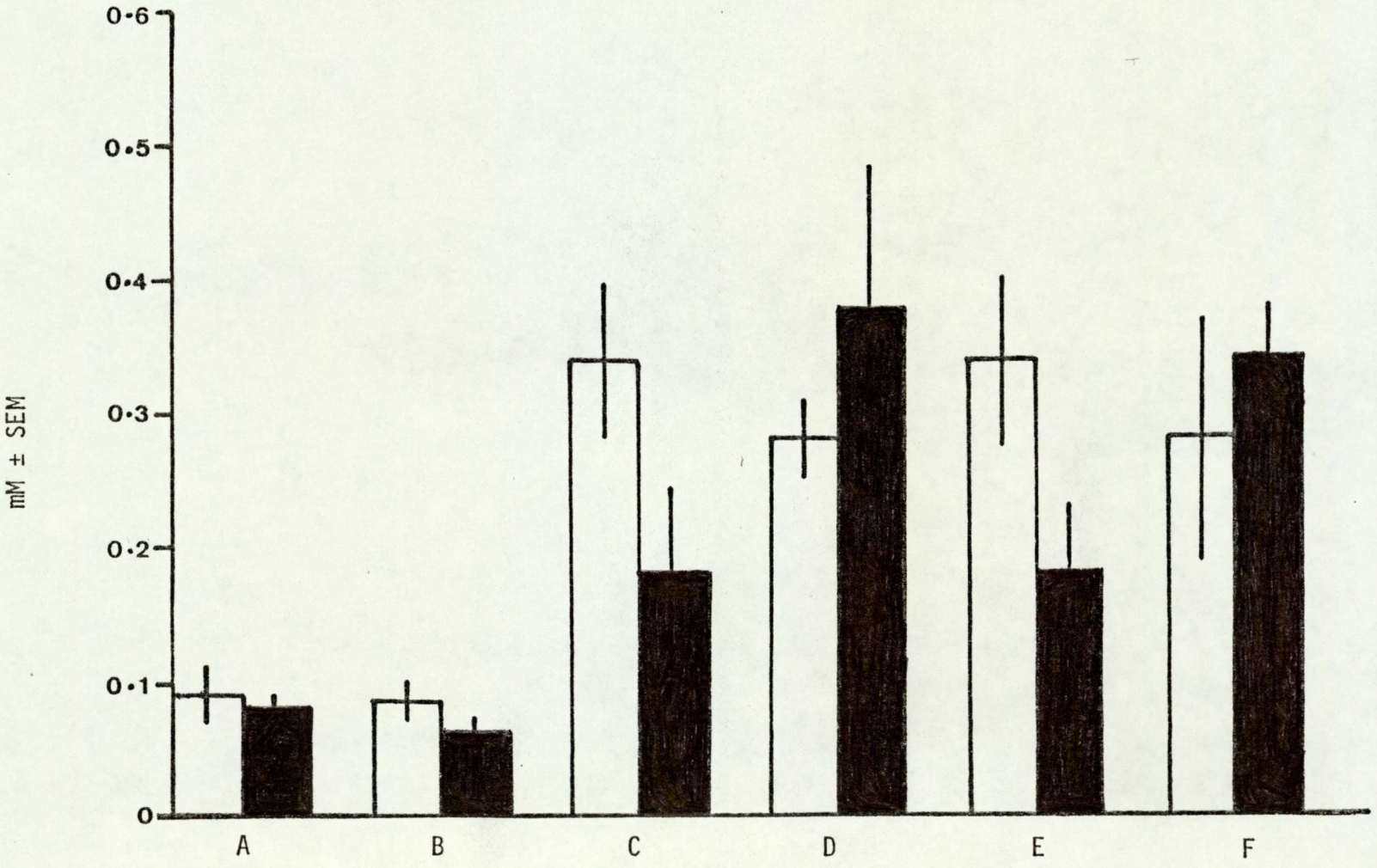


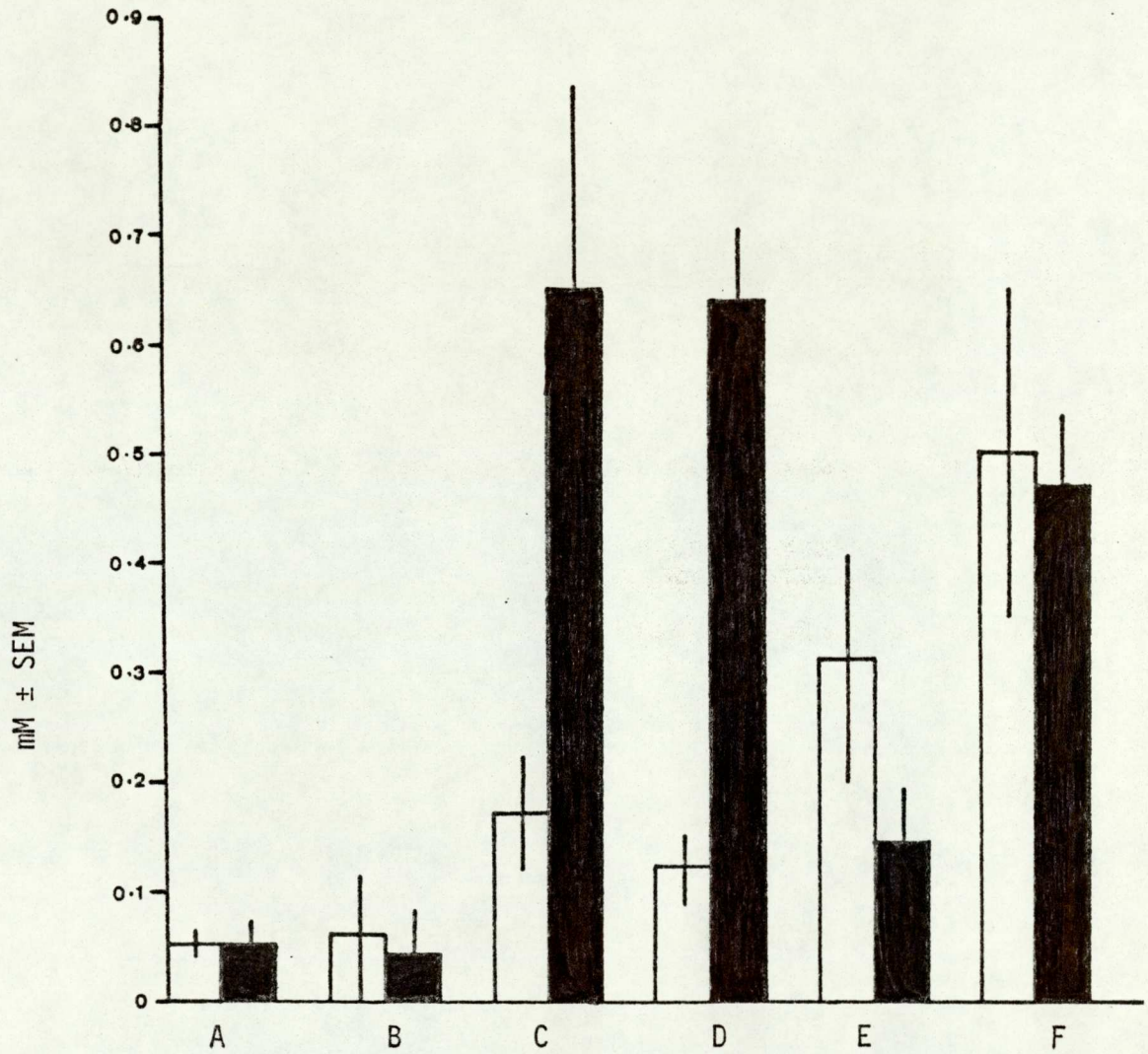
Figure D.3.22 THE EFFECT OF DIETARY MOFIDICATION ON BLOOD ACETOACETATE LEVELS IN FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)

Figure D.3.23 THE EFFECT OF DIETARY MODIFICATION ON LEVELS OF 3-HYDROXYBUTYRATE IN THE BLOOD OF MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key: A Normal diet
B Normal diet + 20% sucrose
C 68% MCT
D 68% MCT + 3OHB
E 80% MCT
F 80% MCT + 3OHB

Figure D.3.24 THE EFFECT OF DIETARY MODIFICATION ON BLOOD ACETOACETATE LEVELS IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B Normal diet + 20% sucrose
C 68% MCT
D 68% MCT + 3OHB
E 80% MCT
F 80% MCT + 3OHB

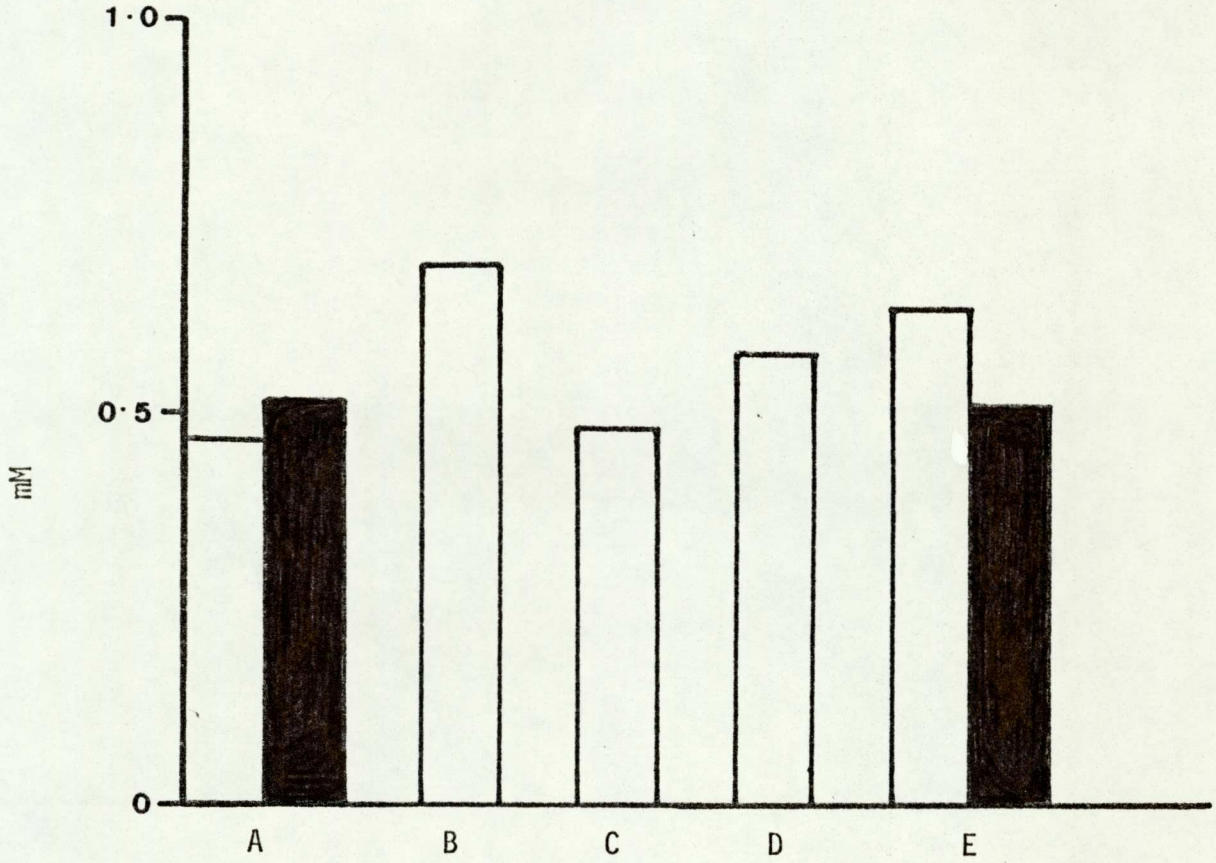
concentrations, when a maximal rate of ketone body utilisation by peripheral tissues is reached (Bates, 1972). The blood concentrations at which this maximum occurs (Bates, 1972) were not reached in this study.

D.3.6.2 Free fatty acid levels

These are shown in Figures D.3.25 and D.3.26 for female and male mice respectively. In the study using female mice, plasma free fatty acid levels were elevated in the groups of tumour-bearing mice fed the 52.5% MCT diet, 68% MCT diet and 68% MCT diet plus 3-hydroxybutyrate compared with those measured in plasma from tumour-bearing and non tumour-bearing mice fed a normal diet. In the study using male mice there was no significant alteration in the levels of circulatory free fatty acids in non tumour-bearing mice fed any of the diets. In the tumour-bearing groups of mice, plasma free fatty acid levels were significantly lower ($p < 0.005$) in mice fed the 68% MCT diet supplemented with 3-hydroxybutyrate compared to levels measured in plasma from tumour-bearing animals fed the normal diet. Levels of free fatty acids were significantly higher ($p < 0.05$) in the tumour-bearing groups of mice fed the 80% MCT diet with and without 3-hydroxybutyrate compared to the corresponding non tumour-bearing groups. Increasing the lipid content of the diet appears to reduce plasma levels of free fatty acids.

D.3.6.3 Plasma insulin levels

Insulin levels were measured in plasma taken from male mice only and the results are displayed in histogram form in Figure D.3.27.

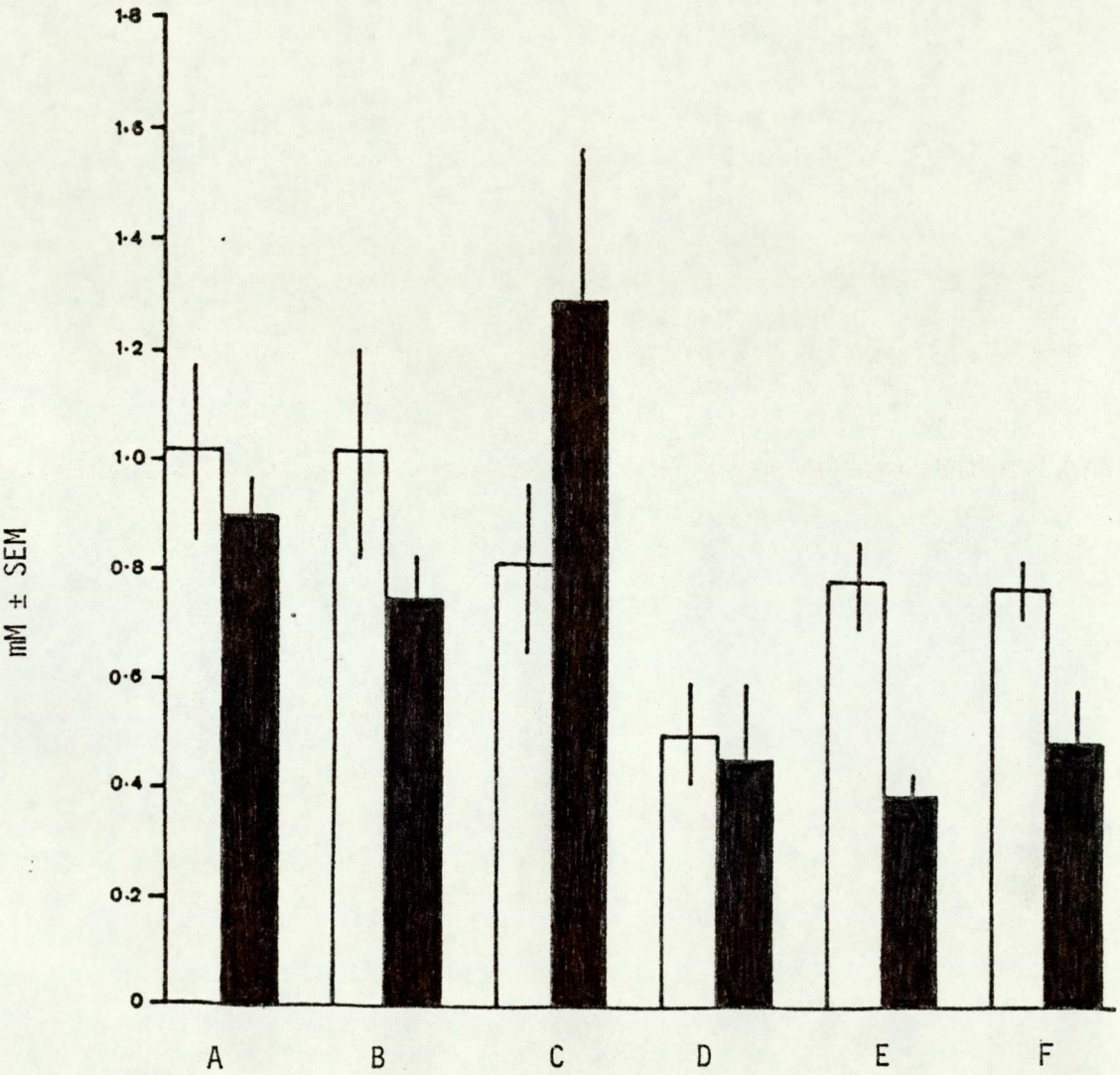


Key:

A Normal diet
B 52.5% MCT
C 52.5% MCT + 3OHB
D 68% MCT
E 68% MCT + 3OHB

Figure D.3.25 THE EFFECT OF DIETARY MODIFICATION ON PLASMA FREE FATTY ACID LEVELS IN FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)

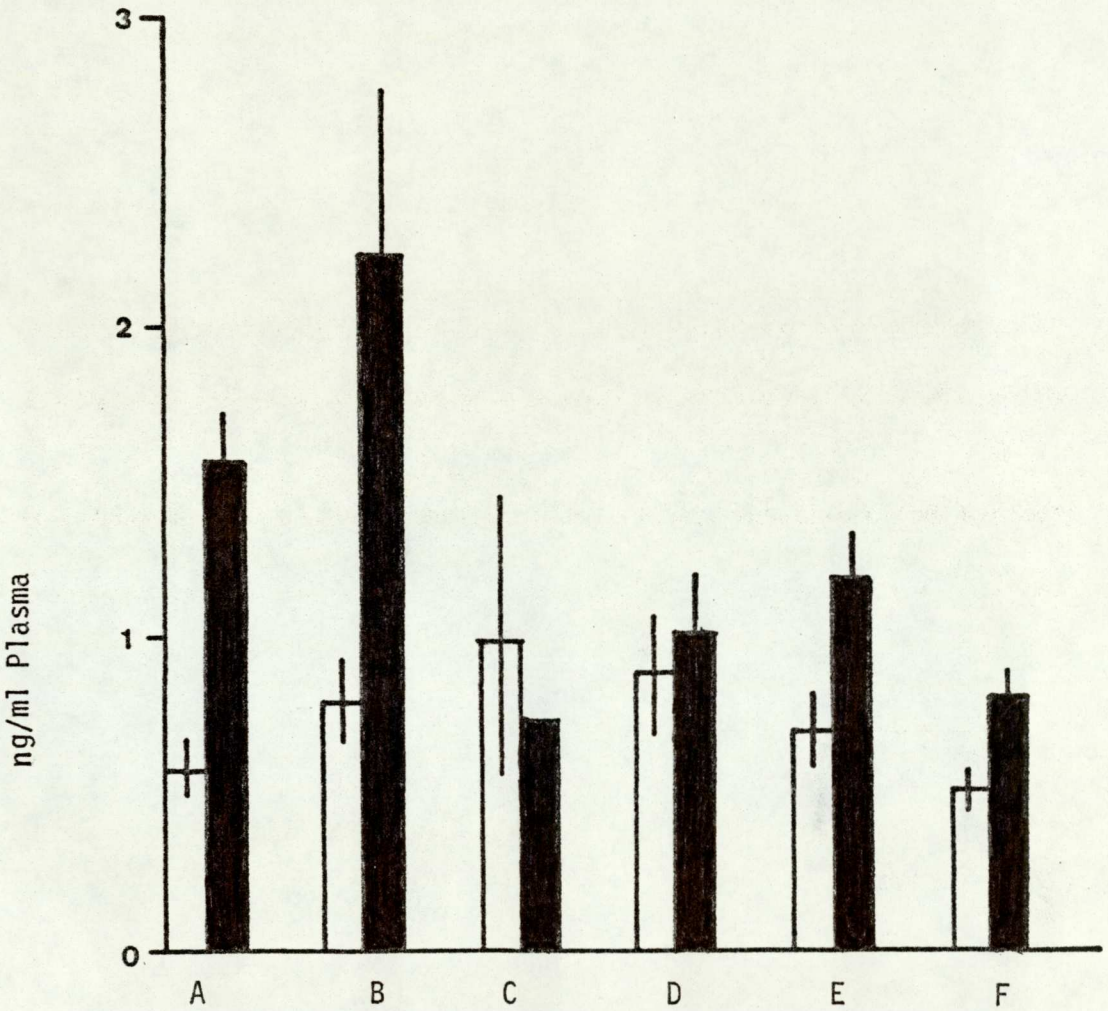
Figure D.3.26 THE EFFECT OF DIETARY MODIFICATION ON PLASMA FREE FATTY ACID LEVELS IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B Normal diet + 20% sucrose
C 68% MCT
D 68% MCT + 3OHB
E 80% MCT
F 80% MCT + 3OHB

Figure D.3.27 THE EFFECT OF DIETARY MODIFICATION ON PLASMA INSULIN LEVELS IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

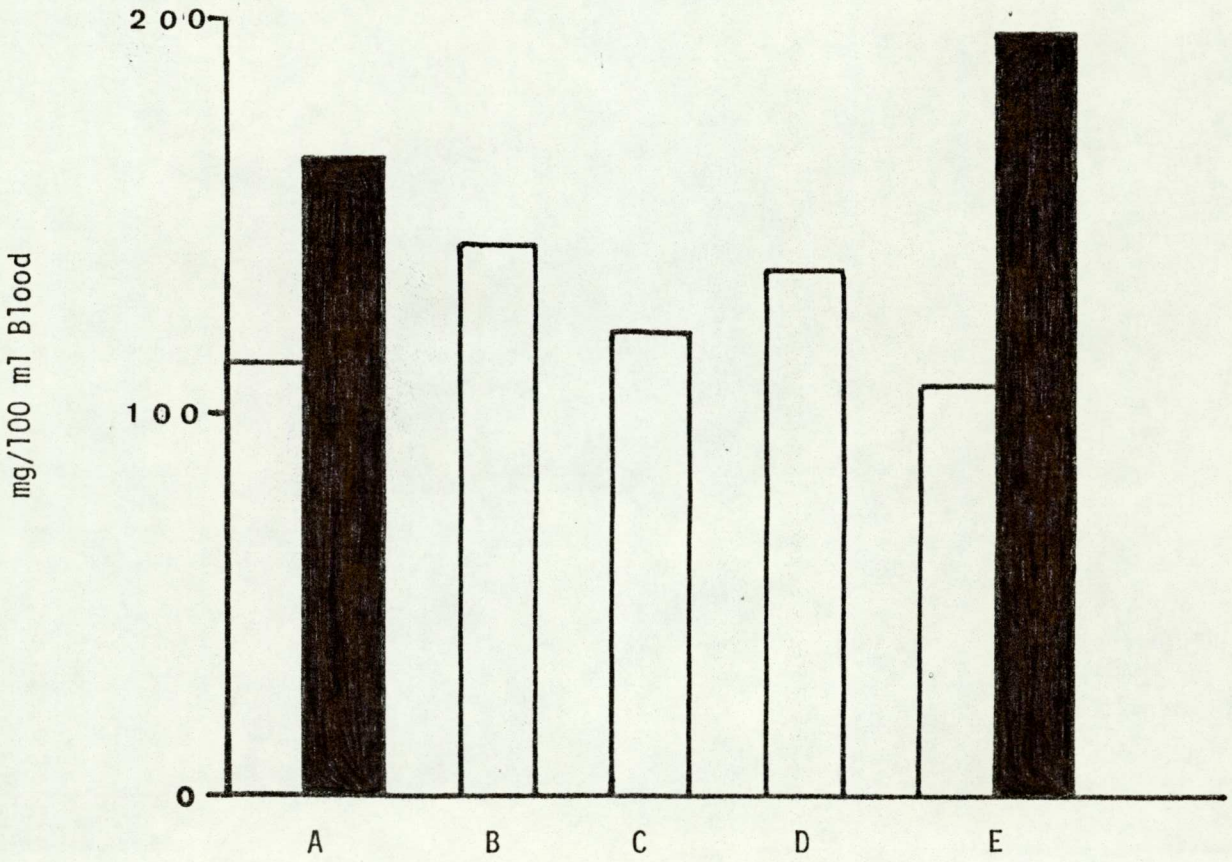
A Normal diet
B Normal diet + 20% sucrose
C 68% MCT
D 68% MCT + 3OHB
E 80% MCT
F 80% MCT + 3OHB

Plasma insulin levels were significantly reduced ($p < 0.05$) in all the groups of tumour-bearing mice with the exception of those fed on the 68% MCT diet, when compared to those measured in the non tumour-bearing groups of mice fed on the normal diet with and without supplementation with 20% sucrose. A reduction in plasma insulin levels also occurred in all the groups of non tumour-bearing mice fed high MCT diets. When tumour-bearing groups are compared with their corresponding control groups, plasma insulin levels were significantly lower ($p < 0.05$) in the groups fed normal diet with and without sucrose, and the 80% MCT diet with and without 3-hydroxybutyrate. Since insulin is known to inhibit lipolysis from adipose tissue, the reduction in plasma insulin levels is presumably associated with the loss of carcass fat seen in the tumour-bearing animals. Inclusion of increasing amounts of triglyceride in the diet had little effect on plasma insulin levels. Plasma insulin levels were significantly higher ($p < 0.05$) in non tumour-bearing mice fed the normal diet supplemented with 20% sucrose compared to all other groups of mice. Sucrose has been shown to stimulate insulinogenesis and insulin release in rats and mice (Blazquez and Lopez Quijada, 1969; Shafrir, 1985). The same effect was not seen in the corresponding tumour-bearing group of mice presumably because of the insulin resistance which is known to occur in tumour-bearing animals (Schien et al, 1979).

D.3.6.4 Blood glucose levels

These results are shown in Figure D.3.28 for the study using female mice and in Figure D.3.29 for the study using male mice. Blood glucose levels in tumour-bearing mice were lower than those in

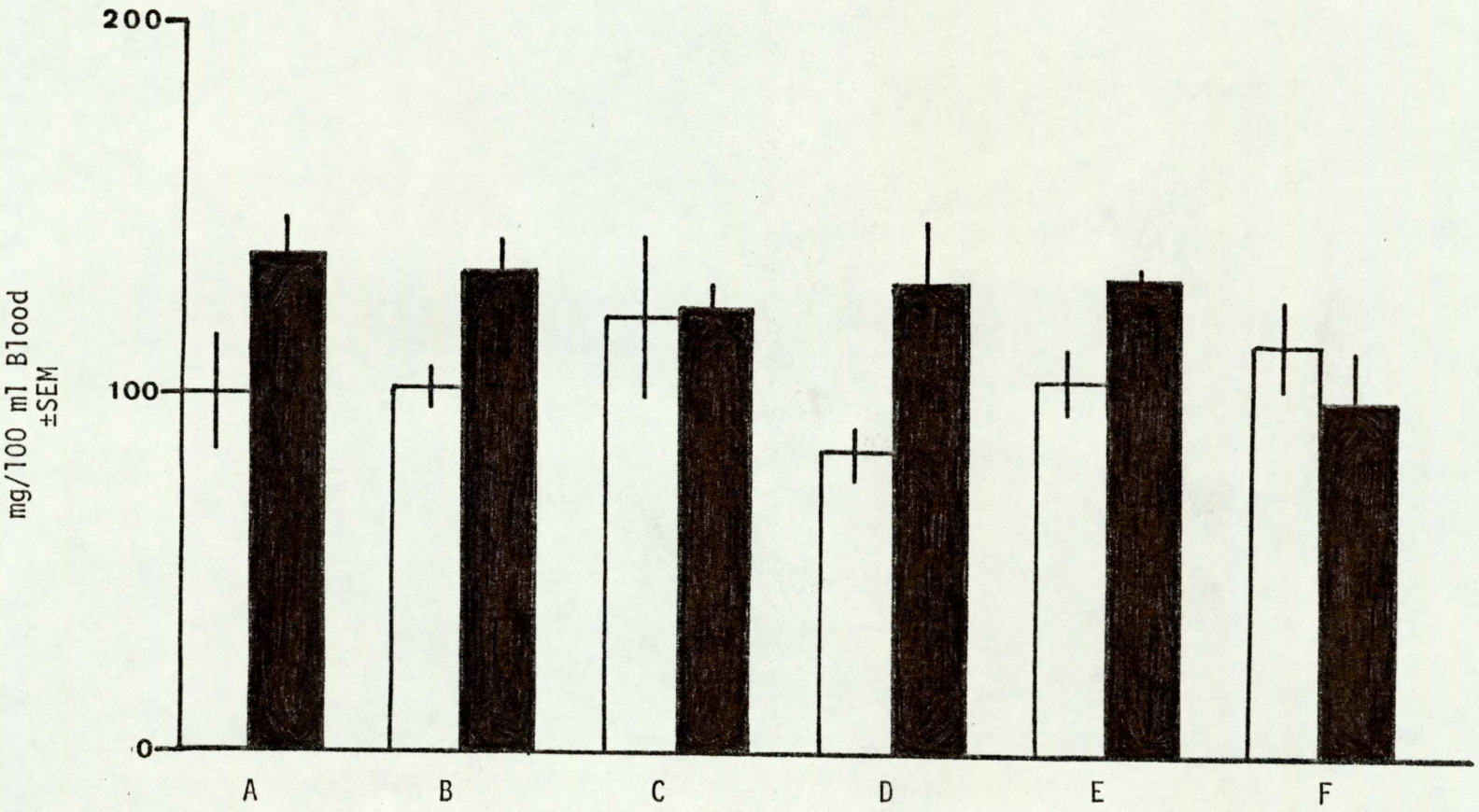
Figure D.3.28 THE EFFECT OF DIETARY MODIFICATION ON BLOOD GLUCOSE LEVELS IN FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B 52.5% MCT
C 52.5% MCT + 3OHB
D 68% MCT
E 68% MCT + 3OHB

Figure D.3.29 THE EFFECT OF DIETARY MODIFICATION ON BLOOD GLUCOSE LEVELS IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:
A Normal diet
B Normal diet + 20% sucrose
C 68% MCT
D 68% MCT + 3OHB
E 80% MCT
F 80% MCT + 3OHB

non tumour-bearing mice in both studies. Levels were significantly lower ($p < 0.01$) in the case of groups fed on the normal diet, normal diet plus 20% sucrose and the 68% MCT diet plus 3-hydroxybutyrate when compared with the corresponding non tumour-bearing diet group. Non tumour-bearing mice fed diets with an increasing proportion of the energy derived from MCT have similar blood glucose levels to those mice fed a normal diet.

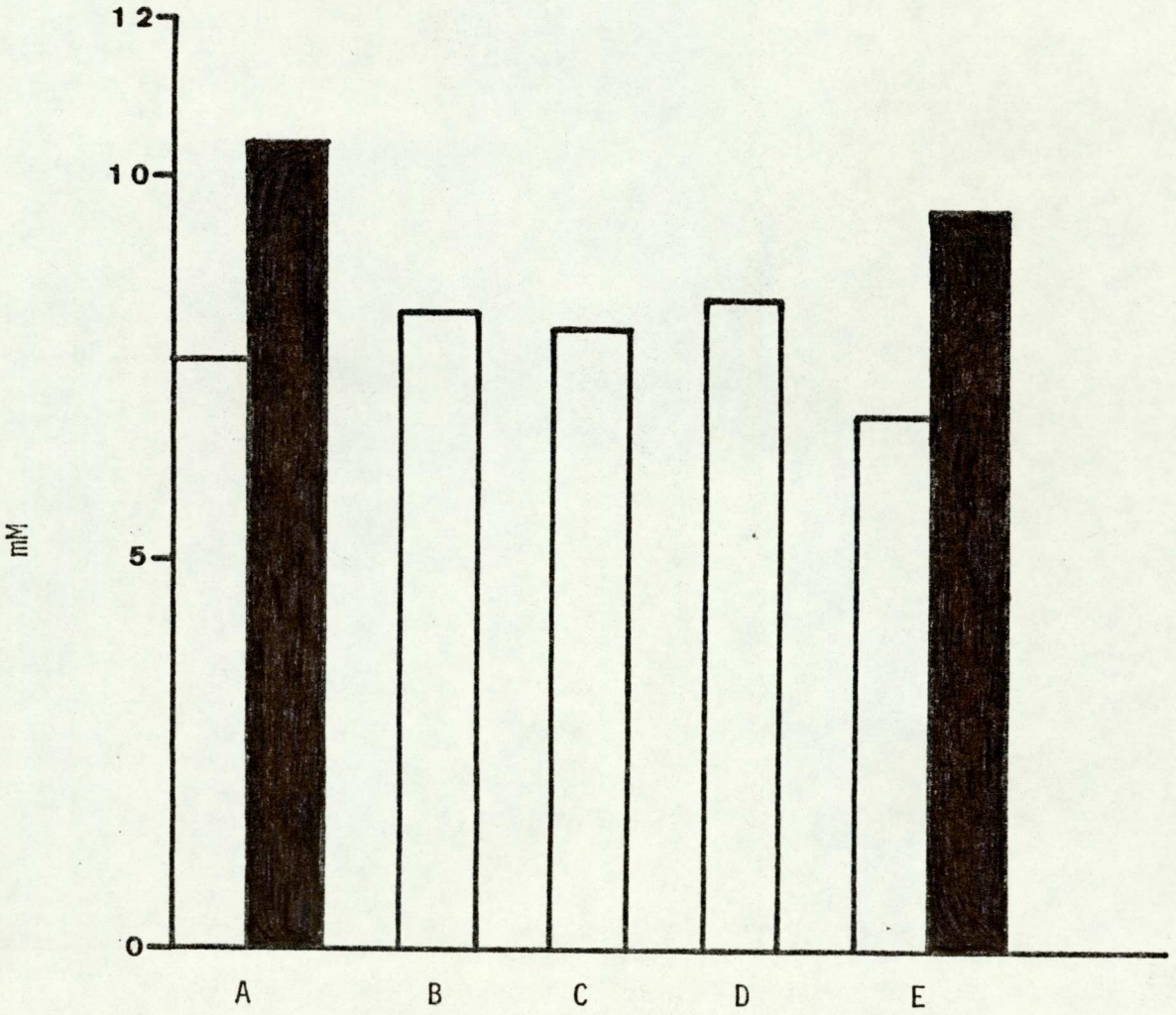
D.3.6.5 Lactate levels

Blood lactate levels are shown in Figures D.3.30 and D.3.31 for female and male mice respectively. Lactate levels in tumour-bearing mice fed the normal diet did not differ significantly from those levels measured in non tumour-bearing mice. The effect of increasing triglyceride levels in the diet is to decrease the blood lactate level although this was only significant ($p < 0.05$) for the tumour-bearing group of mice fed the 68% MCT diet.

D.3.6.6 Amino acid levels

The plasma amino acid levels measured in different diet treatment groups are shown in Table D.3.15 and Figures D.3.32 - D.3.35. Dietary modification and the presence of tumour had little effect on levels of phenylalanine and citrulline. Plasma levels of tyrosine, methionine, arginine, threonine, serine and glycine were reduced in all groups of tumour-bearing mice, and in those groups of non tumour-bearing mice fed high MCT diets. Levels of threonine, serine and glycine were similarly reduced in all the groups fed high MCT diets. The inclusion of triglyceride in the diet led to a

Figure D.3.30 THE EFFECT OF DIETARY MODIFICATION ON BLOOD LACTATE LEVELS IN FEMALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)



Key:

A Normal diet
B 52.5% MCT
C 52.5% MCT + 3OHB
D 68% MCT
E 68% MCT + 3OHB

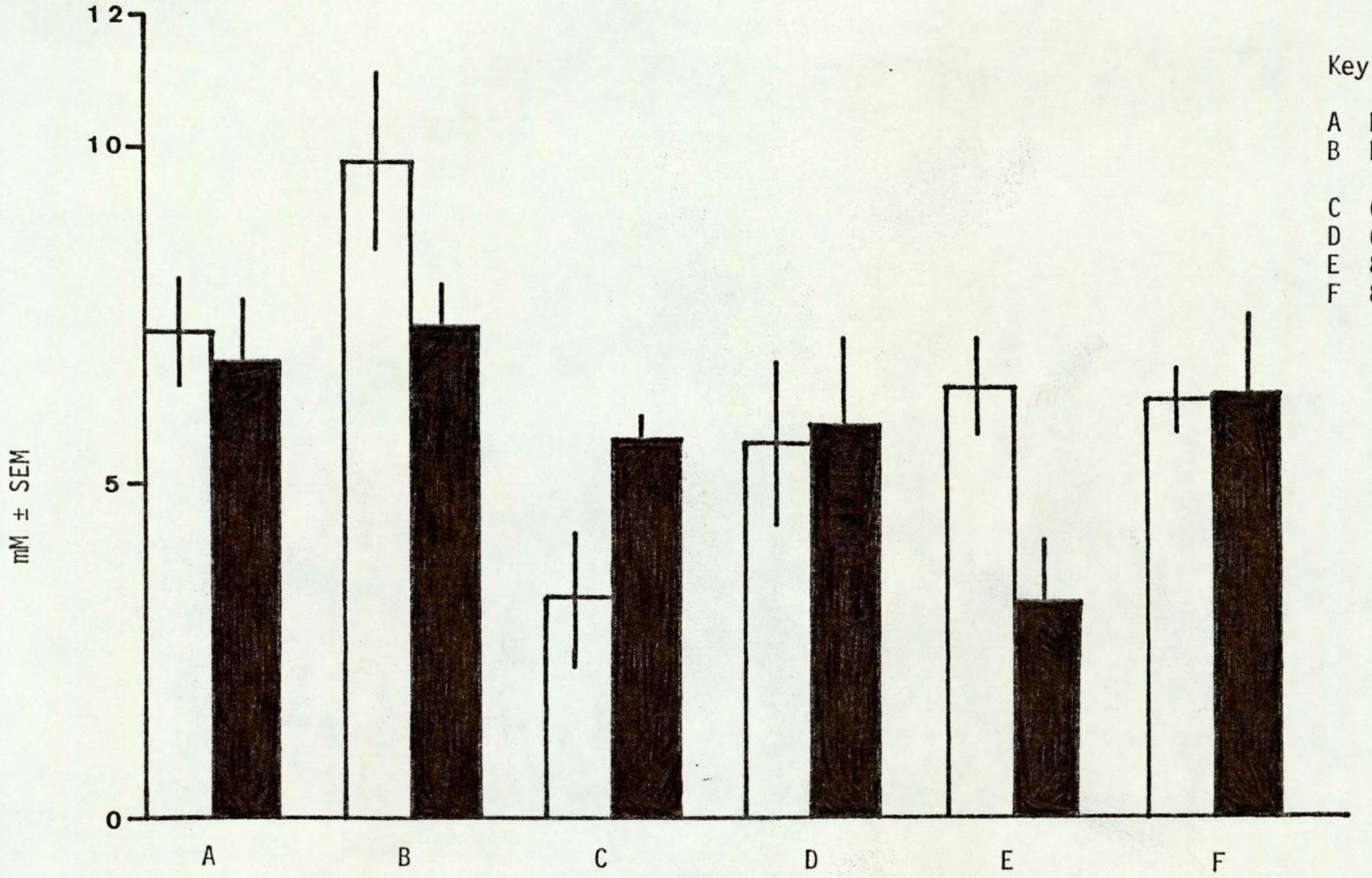


Figure D.3.31 THE EFFECT OF DIETARY MODIFICATION ON BLOOD LACTATE LEVELS IN MALE NMR I MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA (OPEN BOXES) AND NON TUMOUR-BEARING MICE (BLACK BOXES)

Table D.3.20 THE EFFECT OF DIETARY MODIFICATION ON PLASMA AMINO ACID LEVELS IN MALE NMRI MICE IMPLANTED WITH THE MAC 16 ADENOCARCINOMA AND NON TUMOUR-BEARING MICE

AMINO ACID	DIETARY TREATMENT GROUP								
	A	B	C	D	E	F	G	H	I
	NANOMOLES/ML PLASMA								
Taurine	650	560	560	580	530	580	490	430	410
Aspartic acid	12	9	10	5	3	15	8	3	5
Threonine	250	211	220	210	210	270	220	190	210
Serine	190	140	140	150	170	230	150	130	150
Asparagine)									
Glutamic acid)	160	180	190	190	190	200	200	160	150
Glutamine	200	230	190	200	420	150	140	420	390
Glycine	360	260	260	290	310	450	280	230	290
Alanine	570	380	450	430	480	590	500	400	490
Citrulline	70	61	58	62	69	81	58	51	49
α-amino butyric acid		11	12	12	16		13	14	7
Valine	280	410	450	390	480	360	360	360	380
Methionine	61	56	56	55	59	86	64	62	61
Isoleucine	120	190	200	180	200	140	170	170	170
Leucine	190	280	290	250	310	230	240	230	240
Tyrosine	85	95	110	71	100	120	100	89	88
Phenylalanine	100	120	120	110	120	120	120	100	100
β-aminobutyric	5	5	5	5		6	6		
β-alanine	1	1	2	2		3	3		
Ornithine	270	260	260	250	420	220	220	180	200
Lysine)									
Tryptophan)	560	450	410	380	220	610	440	250	260
)					100				82
Histidine	89	91	94	100	90	100	110	120	110
1-Methylhistidine	6	1	1	1		4			
3-Methylhistidine	5	4		3		3			
Arginine	48	28	47	35	25	190	89	46	67

Table D.3.20 continued

Key:	Tumour-bearing mice	Group A	Normal diet
		Group B	68% MCT
		Group C	68% MCT + 3-hydroxybutyrate
		Group D	80% MCT
		Group E	80% MCT + 3-hydroxybutyrate
	Non Tumour-bearing mice	Group F	Normal diet
		Group G	68% MCT + 3-hydroxybutyrate
		Group H	80% MCT
		Group I	80% MCT + 3-hydroxybutyrate

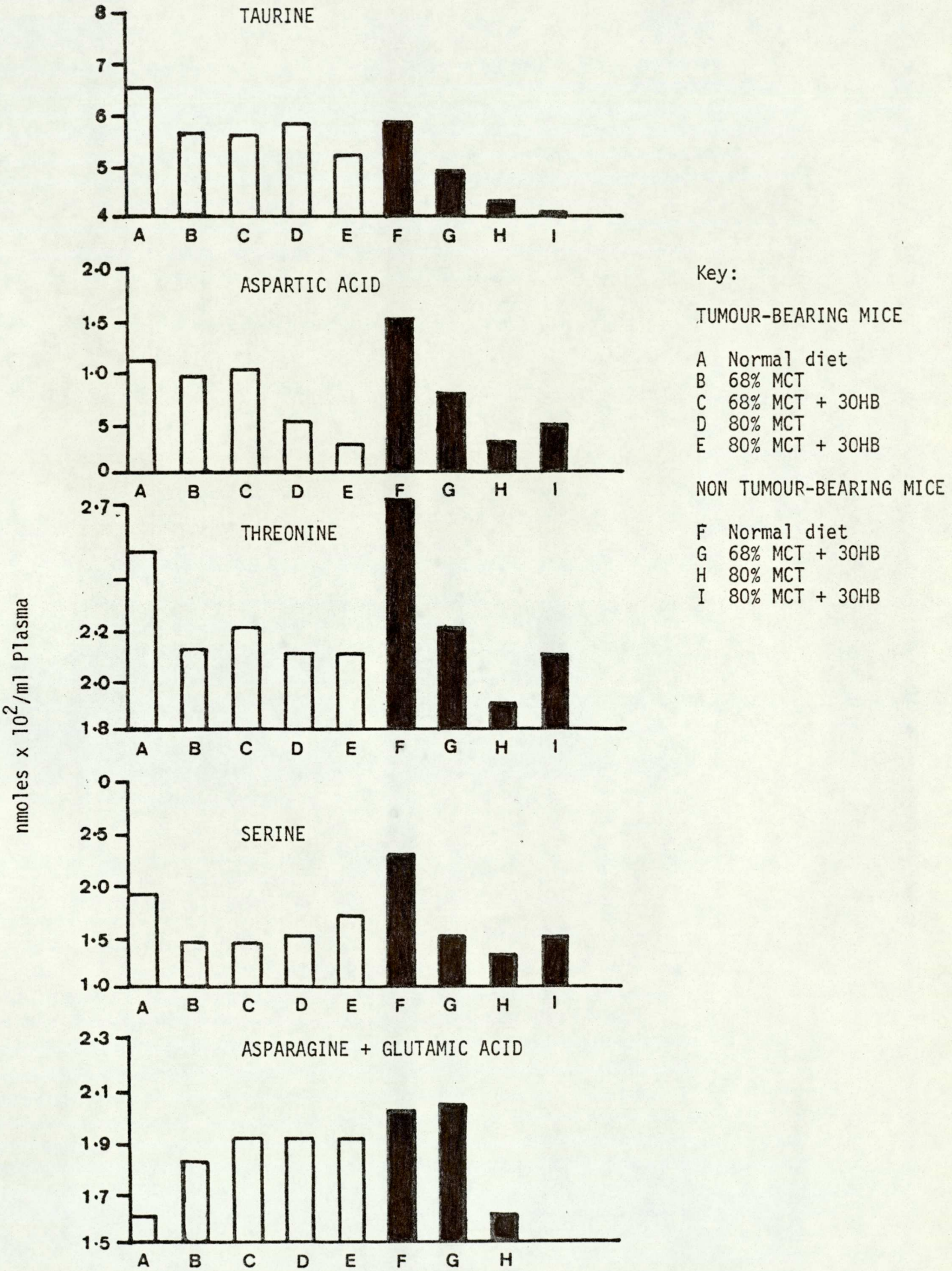


Figure D.3.32 THE EFFECT OF DIETARY MODIFICATION ON PLASMA AMINO LEVELS IN MALE NMR I MICE.

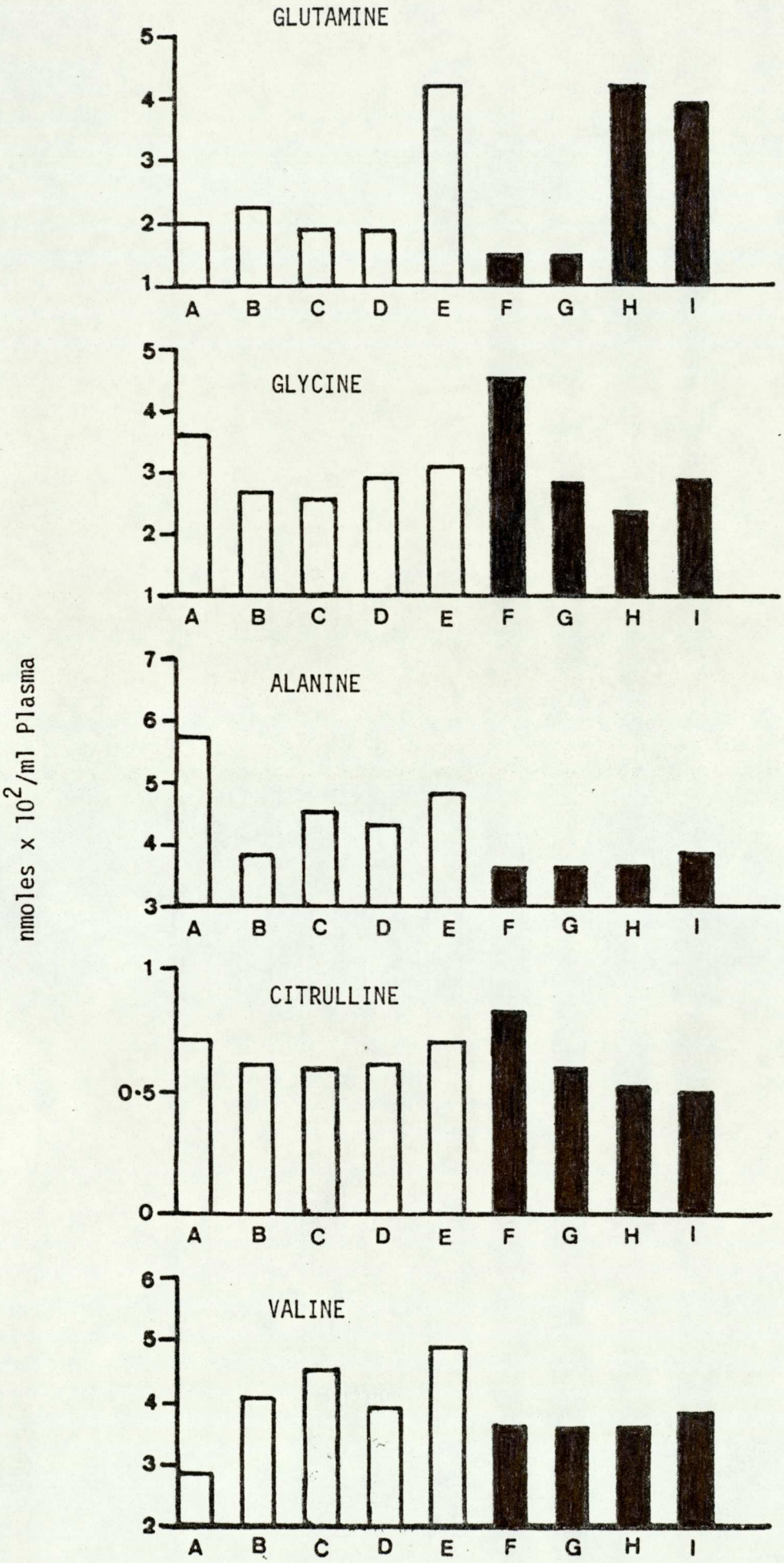


Figure D.3.33 THE EFFECT OF DIETARY MODIFICATION ON PLASMA AMINO ACID LEVELS IN MALE NMR I MICE

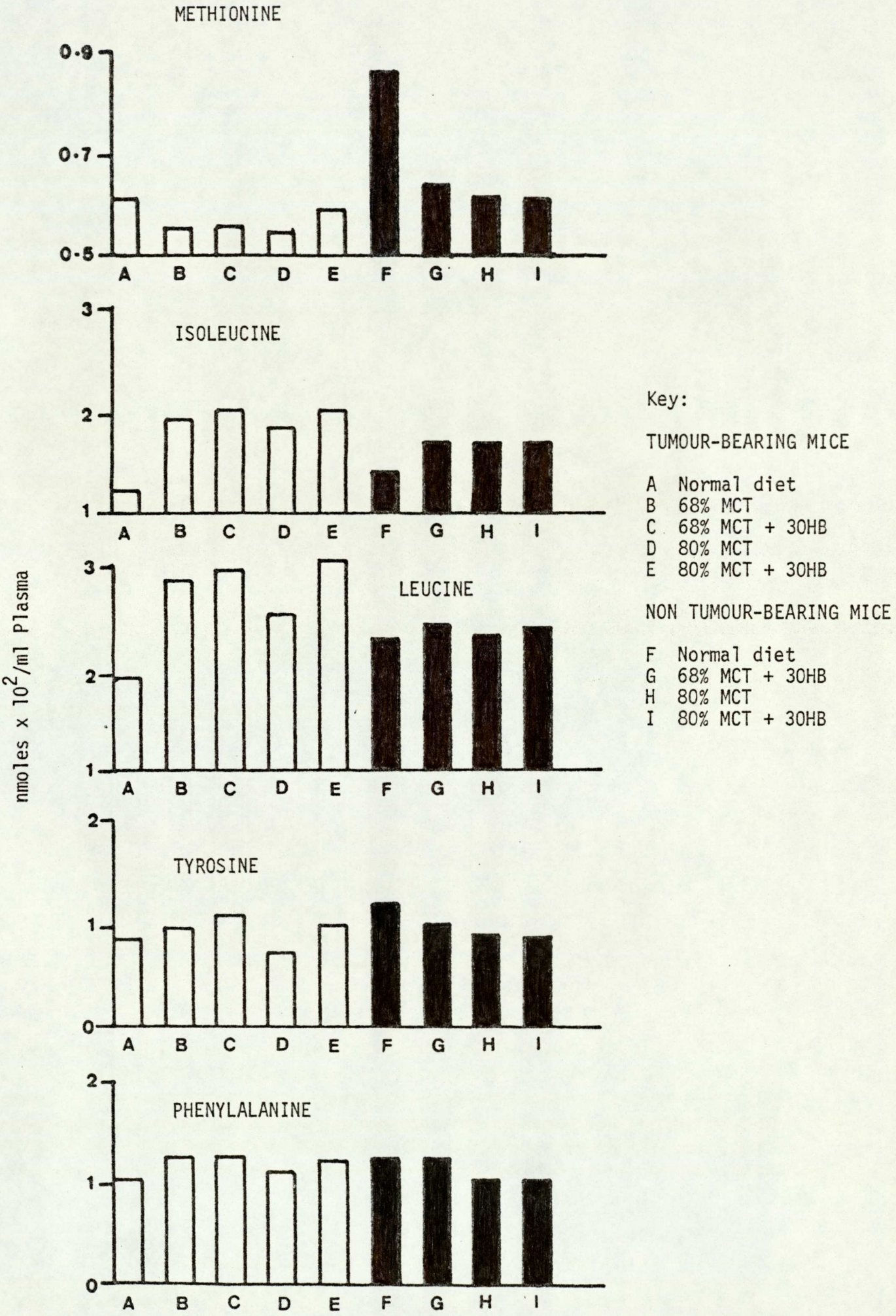


Figure D.3.34 EFFECT OF DIETARY MODIFICATION ON PLASMA AMINO ACID LEVELS IN MALE NMR I MICE

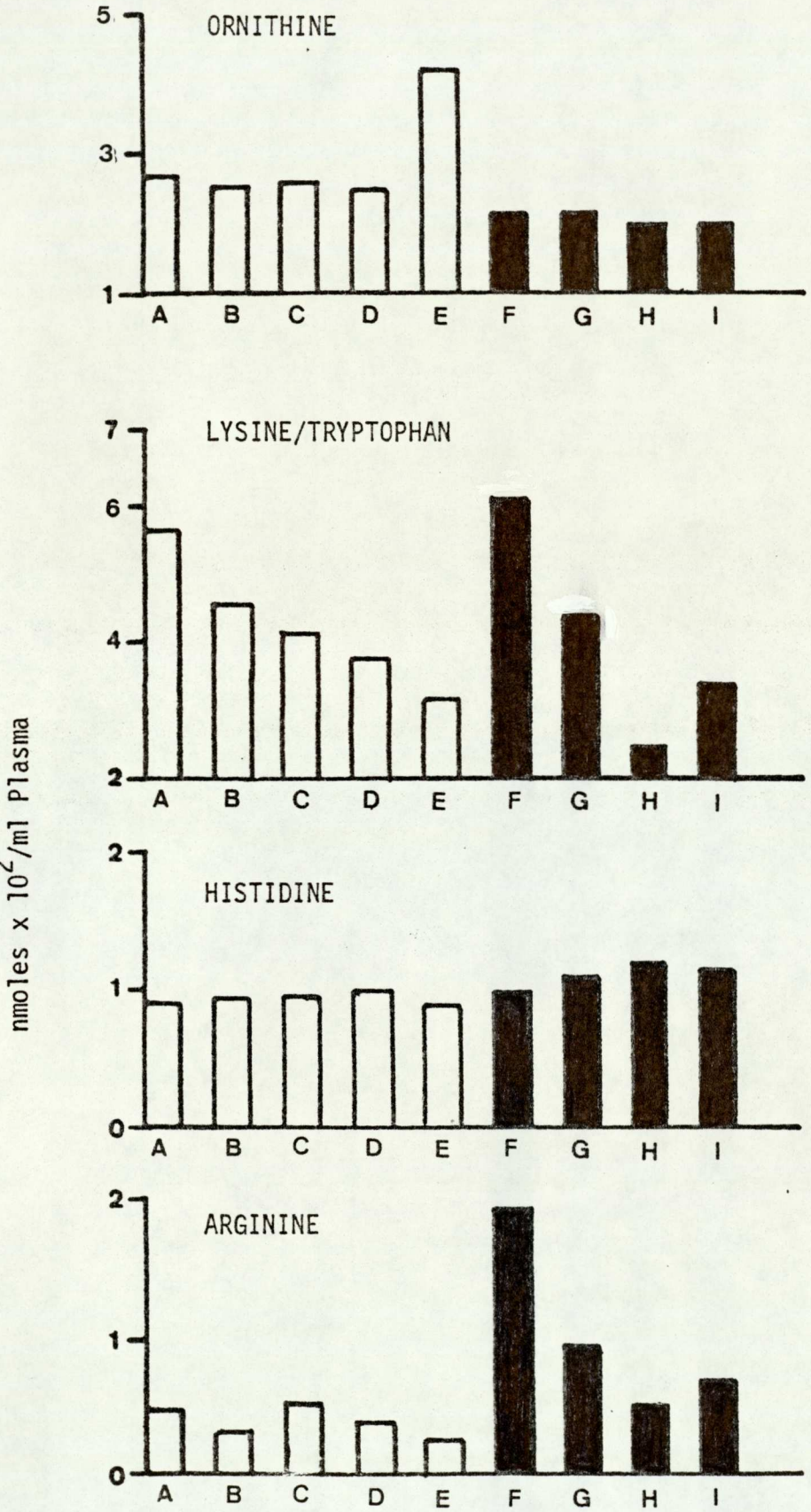
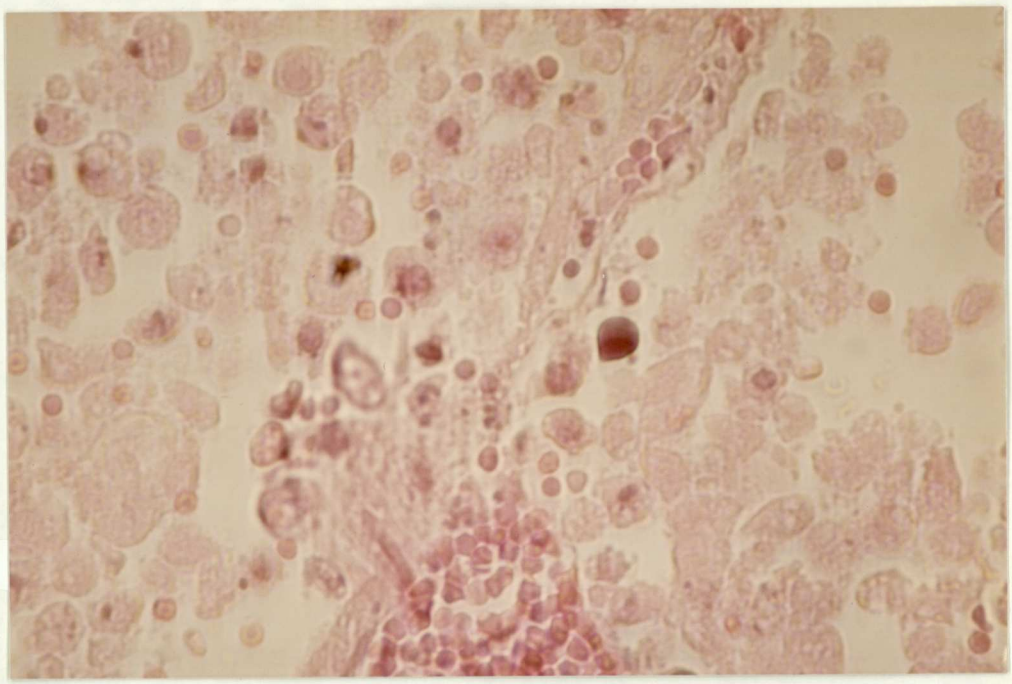


Figure B.3.35 THE EFFECT OF DIETARY MODIFICATION ON PLASMA AMINO ACID LEVELS IN MALE NMR I MICE

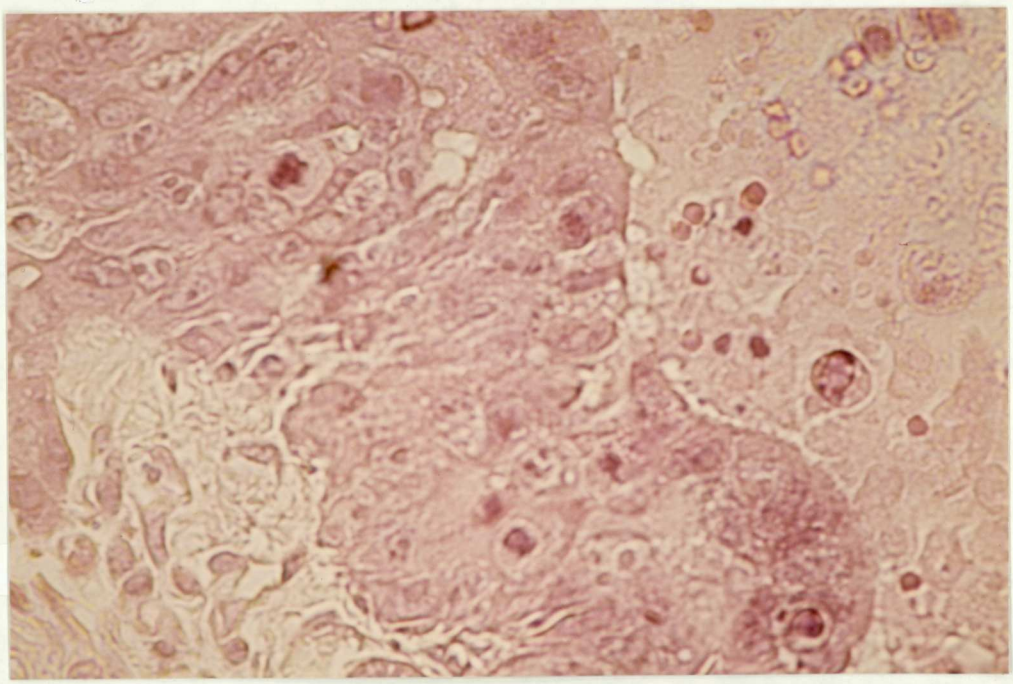
reduction in levels of aspartic acid, lysine, tryptophan and alanine irrespective of the presence of tumour. The branched-chain amino acids valine, isoleucine and leucine were reduced in tumour-bearing mice fed a normal diet, and increased in all the tumour-bearing groups fed high MCT diets. Levels of isoleucine were also increased in non tumour-bearing groups of mice fed high MCT diets. Glutamine levels were elevated in all groups of tumour-bearing mice fed the 80% MCT diet with and without 3-hydroxybutyrate. Plasma levels of taurine were elevated in the group of tumour-bearing animals fed on the normal diet, and decreased in all the non tumour-bearing groups fed diets with a high lipid content. The presence of tumour and diet were found to influence plasma amino acid levels. Increasing the proportion of fat in the diet counteracted the effects of the presence of tumour in the case of some of the amino acids, presumably as a result of the increase in ketone body levels.

D.3.7 The effects of dietary modification on the histological structure of the MAC 16 adenocarcinoma

Figures D.3.36 - D.3.40 show the histological structure of cross-sections of tumours taken from animals in the different diet treatment groups at the end of the study. Cross-sections of tumour taken from mice fed on a normal diet are shown in Figures D.3.36 and D.3.37, and show extensive necrosis and the presence of very few blood vessels. Increasing the proportion of triglyceride in the diet reduces the areas of necrosis and increases the degree of vascularization in the tumour (Figures D.3.38 - D.3.40). These changes in morphological structure seen in tumours from animals fed on the high MCT diets with and without supplementation with 3-hydroxybutyrate for



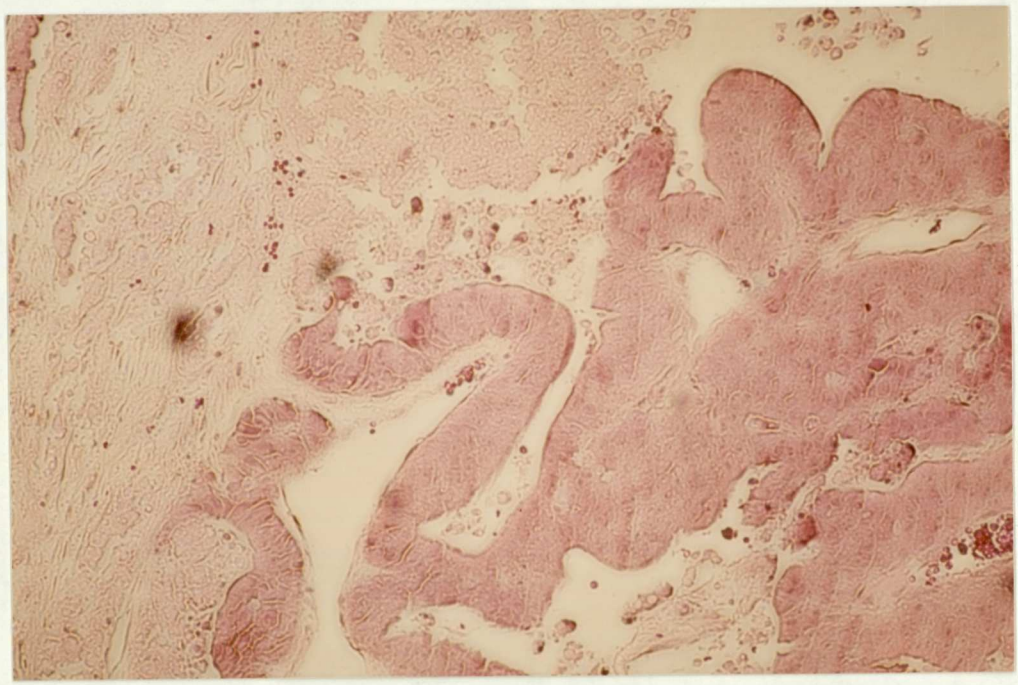
a) x 50 mag.



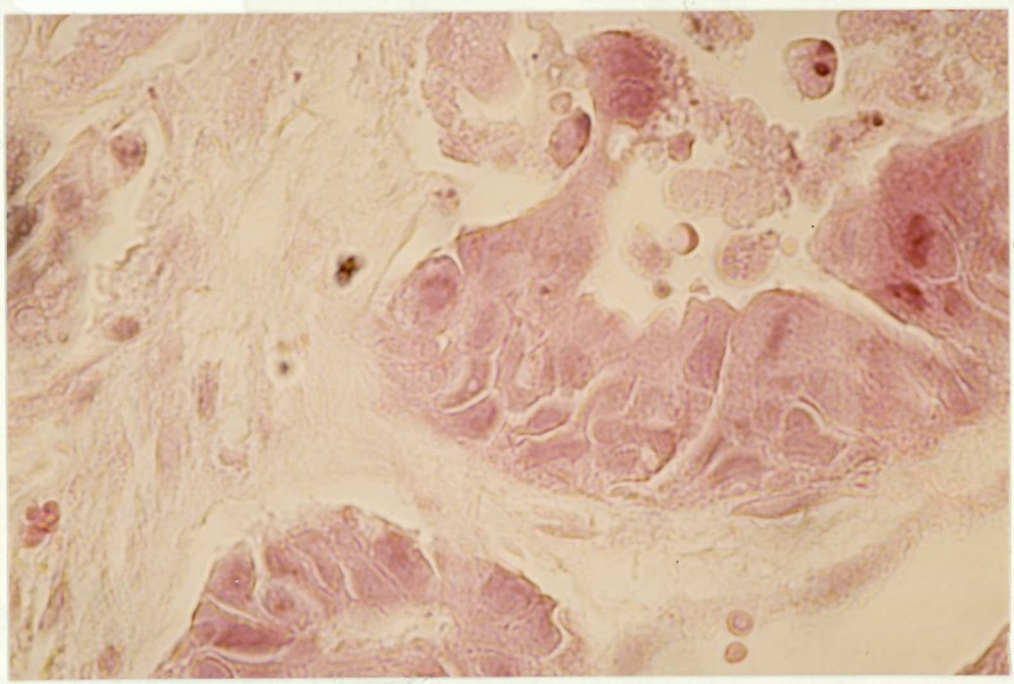
b) x 200 mag

Figure D.3.36

a) Section through the MAC 16 adenocarcinoma from mice fed the normal diet. The tumour is poorly vascularised and shows extensive necrosis.
b) Higher magnification (x200) showing acellular regions and necrosis.



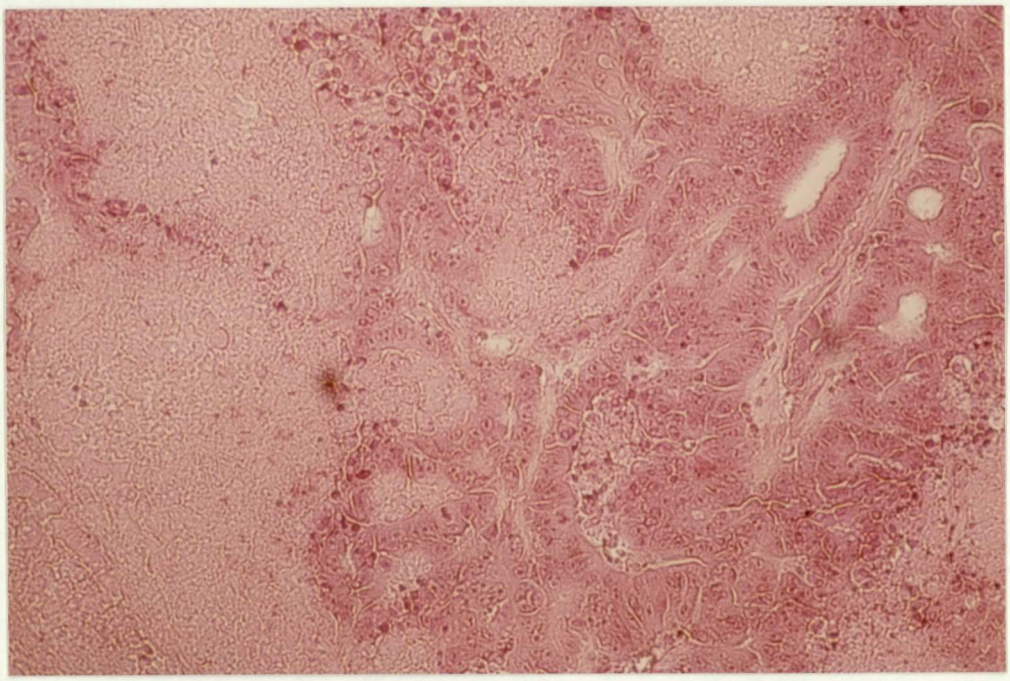
a) x 50 mag.



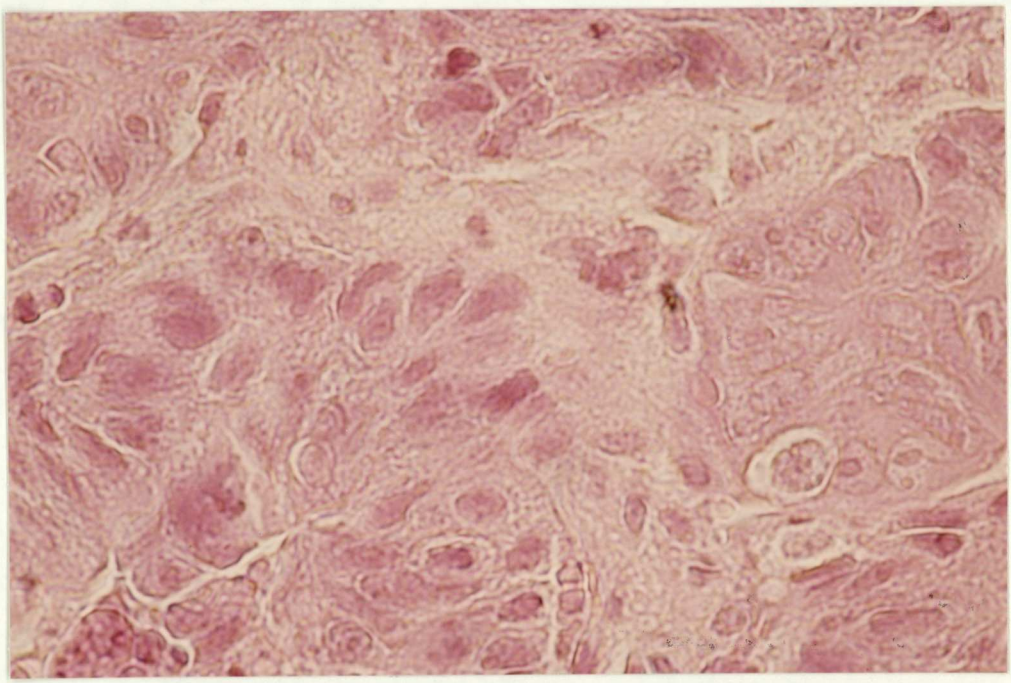
b) x 200 mag.

Figure D.3.37

Section through the MAC 16 adenocarcinoma from mice fed the normal diet supplemented with 20% sucrose.



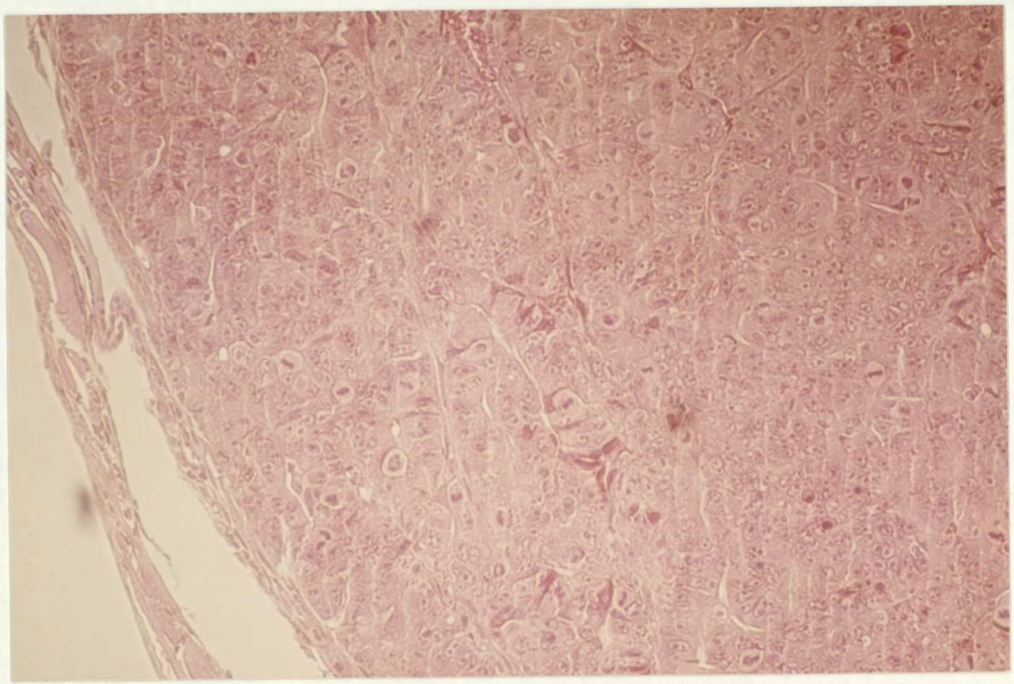
a) x 50 mag.



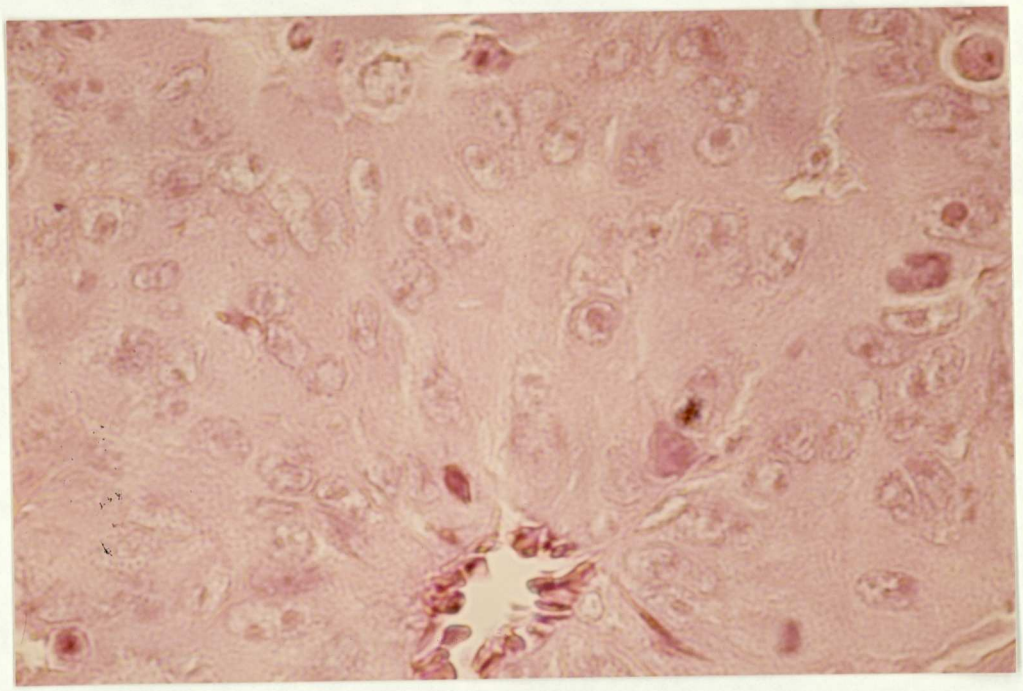
b) x 200 mag.

Figure D.3.38

Section through the MAC 16 adenocarcinoma from mice fed on the 68% MCT diet supplemented with 3-hydroxybutyrate arginine salt. Areas of necrosis are reduced and there is an increase in vascularisation of the tumour.



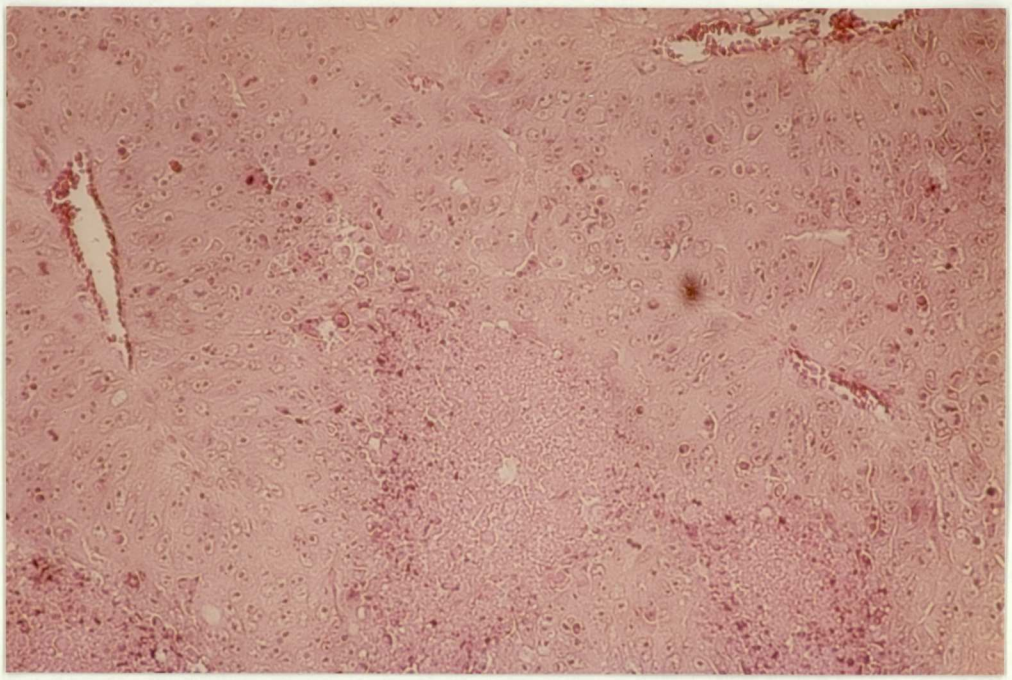
a) x 50 mag.



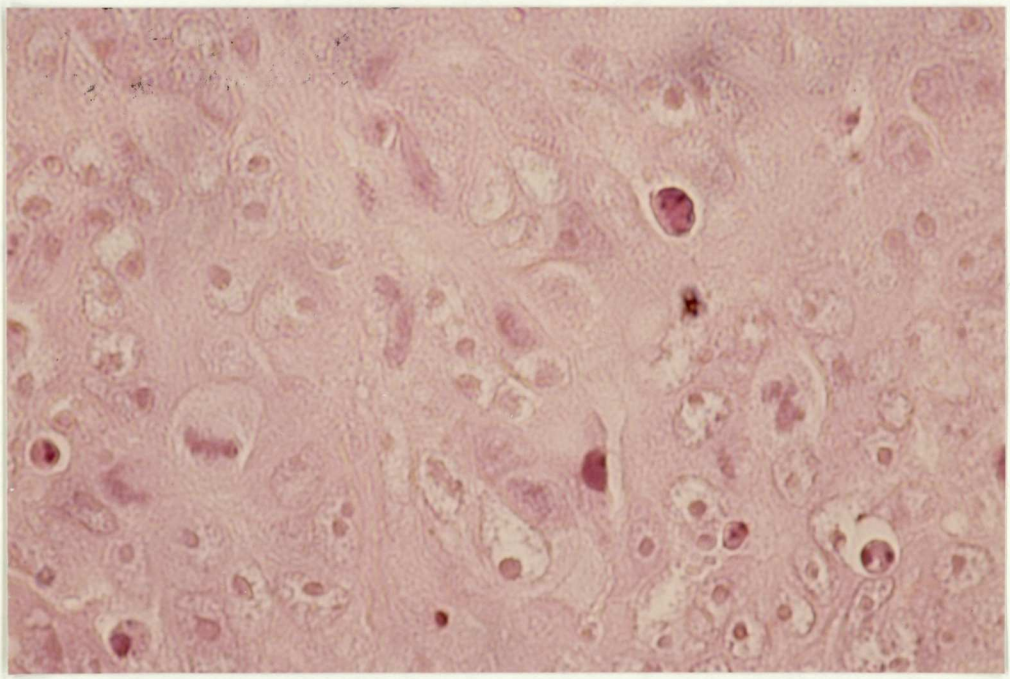
b) x 200 mag.

Figure D.3.39

Section through the MAC 16 adenocarcinoma from mice fed on the 80% MCT diet. The areas of necrosis are significantly decreased and there is a marked increase in the degree of vascularisation.



a) x 50 mag.



b) x 200 mag.

Figure D.3.40

Section through the MAC 16 adenocarcinoma from mice fed on the 80% MCT diet supplemented with 3-hydroxybutyrate arginine salt. The structure is similar to that of the tumour taken from mice fed on the 80% MCT diet alone.

24 days, are presumably an attempt by the tumour to reoxygenate itself in order to use dietary lipids which cannot be utilised in the absence of oxygen. These changes, however, would also make the tumour more vulnerable to chemotherapy and radiotherapy which are largely ineffective in the treatment of tumours which are poorly vascularized and hypoxic.

E. Discussion

E Discussion

The MAC 16 adenocarcinoma, which is one of a series of colonic tumours originally induced in NMR I mice by dimethylhydrazine (Double et al, 1975), is perhaps one of the best animal tumour models available for the study of cachexia. Most models of cachexia have utilised rapidly growing transplantable rodent tumours that cause the symptoms of cachexia to begin only at the later stages of tumour growth, when the tumour may represent 30-40% of the total body weight (Strain et al, 1980). This is quite unlike what happens in the human situation in which tumour growth is slow and tumour burden rarely exceeds 5% of the body weight before death (Waterhouse, 1974; Costa, 1977).

The MAC 16 adenocarcinoma is relatively slow growing, taking around 28 days to reach a size of around 2 grams, which represents about 10% of the host's body weight. At this time the tumour is centrally necrotic and has produced extensive weight loss of approximately 20% in the tumour-bearing animal. Furthermore, the tumour does not have to reach an extensive mass in order to produce weight loss. The results of this study have shown that there is a significant weight loss in tumour-bearing animals 14 days after implantation with tumour, at which time the tumour mass is only approximately 0.3 gram, which is less than 1% of the host body weight. This weight loss can be clearly seen as a reduction in the size of the animal and in the disappearance of fat deposits (Figure D.2.5). No evidence of ketosis was found in animals fed on the normal pelleted food, and weight loss was not accompanied by a reduction in food intake, which is in agreement with the findings of Ali et al (1985) and Dr K H Fearon (personal communication). The

MAC 16 adenocarcinoma is therefore considered to be an appropriate model of human cachexia where weight loss has a metabolic rather than anorectic origin, and has thus made a study of the cachectic syndrome possible.

The mechanisms by which the tumour produces cachexia have been the subject of many investigations and a number of hypotheses have been put forward, some of which were investigated in this study. One line of investigation undertaken in this study arose from the observation that ketosis is essentially absent in the cachectic cancer patient (Conyers et al, 1979a), a feature which is thought to explain some of the characteristics of cancer cachexia, and that it might be possible to exploit this feature in devising a suitable means of therapy to counteract this condition. Ketone bodies are an important source of metabolic fuel for the tissues in place of glucose, and they also play an important role in regulating metabolism (Cahill, 1970; Sherwin et al, 1975; Robinson and Williamson, 1980). The utilisation of ketone bodies by the tissues requires the presence of three enzymes, 3-hydroxybutyrate dehydrogenase, 3-oxo acid-CoA transferase and acetoacetyl-CoA thiolase. A previous study in which the levels of activity of these enzymes were measured in the tissues of Balb/c mice and in a number of tumours of peripheral tissues (Tisdale and Brennan, 1983) showed that one of the enzymes, 3-oxo acid-CoA transferase which catalyses the conversion of acetoacetate to acetoacetyl-CoA, was virtually absent in many of the tumours examined. This result indicated that these tumours might be unable to utilise ketone bodies as a metabolic fuel and suggested that it might be possible to selectively starve such tumours using dietary means. In the present study the levels of activity of the three enzymes involved in the utilisation of ketone bodies were

measured in the tissues of NMR I mice, both with and without the MAC 16 tumour. The distribution of these enzymes was found to be similar to those reported for Balb/c mice. The level of activity of 3-oxo acid-CoA transferase measured in MAC 16 tumour excised from NMR I mice was lower than levels of activity found in the other tissues and in particular the normal colon which is comparable to the MAC 16 histologically. The only exception to this was the liver, a tissue not regarded as having the ability to use ketone bodies as metabolic substrates (Williamson et al, 1971).

Levels of activity of 3-oxo acid-CoA transferase measured in the direction of acetoacetate formation, referred to as the back reaction, were much higher than levels measured in the direction of acetoacetyl-CoA formation. This finding was in agreement with those of Williamson et al (1971), and Fenselau and Wallis (1974). Stern et al (1956) studied the properties of 3-oxo acid-CoA transferase, and found that the enzyme catalysed the transfer of CoA from acetoacetyl-CoA to succinate more rapidly than the reverse reaction. They determined the K_m values for each of the substrates involved in the reactions, and found that the K_m value for acetoacetyl-CoA was lower than those obtained for the other substrates, succinyl-CoA, acetoacetate and succinate. This finding indicated that 3-oxo acid-CoA transferase has a greater affinity for acetoacetyl-CoA than for the other substrates, therefore providing an explanation for the difference in the levels of activity of the enzyme measured in the forward and backward directions. The low levels of activity of 3-oxo acid-CoA transferase measured in the MAC 16 adenocarcinoma suggest that this tumour may possess a limited capacity to utilise ketone bodies as metabolic substrates.

The levels of a number of metabolites were measured in the blood

of NMR I mice with and without the MAC 16 adenocarcinoma. Insulin levels fell as a result of the presence of tumour, and since insulin has a regulatory effect on metabolism, low levels promoting increased mobilisation of glucose, free fatty acids and amino acids from the tissues, this reduction could explain some of the catabolic effects seen in cachexia. In studies with tumour-bearing rats, Chance et al (1983) found that reduced levels of plasma insulin resulted in the maintenance of normal blood glucose levels. In the present study, normal blood glucose levels were maintained in tumour-bearing mice until day 14 after implantation with tumour. By day 21, however, blood glucose levels were significantly lower than those in non tumour-bearing mice. The depletion of body fat which has been shown to occur with the growth of the tumour may also be the result of a fall in plasma insulin levels, since insulin has been shown to be a potent inhibitor of lipolysis. Insulin inhibits lipolysis possibly by lowering adipose cell cyclic AMP levels, thereby decreasing the activity of the lipase responsible for initiating triglyceride hydrolysis (Butcher, 1968). Plasma free fatty acid concentrations in tumour-bearing mice were found to be only slightly higher than those in the control mice initially, but decreased to levels which were slightly lower than those measured in control mice at the end of the study when tumour size was large. A fall in plasma free fatty acid levels in tumour-bearing animals might be expected as the tumour increases in size, and the fat stores of the body gradually become exhausted, particularly as the results of this study have shown that the decrease in total carcass fat content, which occurred in mice bearing the MAC 16 tumour, was proportional to the size of the tumour.

The cyclic metabolic pathway in which glucose is converted to

lactate and then lactate reconverted back to glucose again is termed the Cori cycle, and this has been found by some workers to be abnormally high in the cachectic cancer patient (Waterhouse, 1974; Holroyde et al, 1975). Utilisation of lactate by the liver for glucose synthesis is an energy-requiring process requiring 6 moles of ATP derived from normal host sources per mole of glucose synthesised and utilised by the tumour. The anaerobic breakdown of glucose to lactic acid by the tumour yields 2 moles of ATP, for a potential net loss of 8 moles of high energy phosphate by normal tissues. The increased rate of conversion of lactate to glucose via the Cori cycle has been proposed as a mechanism for the increased energy expenditure of the tumour-bearing patient (Fenninger and Mider, 1954; Gold, 1974). Some tumours show high rates of anaerobic glycolysis with the production of lactic acid (Warburg, 1930; Gold, 1966). This has also been demonstrated in vitro with the MAC 16 cell line grown in tissue culture (Tisdale and Brennan, 1986). Studies in cancer patients have also shown increased lactate production; Holroyde et al (1979) showed that lactate production was significantly increased in patients with metastatic colorectal cancer compared to healthy non tumour-bearing controls. In the present study, blood lactate levels in tumour-bearing mice were similar to those measured in non tumour-bearing mice. An explanation for this may be that put forward by Schein and co-workers (1979), who made the observation that increased lactate production in most cancer patients could not be detected by simple measurement of blood levels of this metabolite at a specific time-point. They suggested that it was possible that the magnitude of the expanded lactate pool was such that it could be readily consumed by an accelerated gluconeogenic activity, which would serve to maintain blood concentration within the normal range.

Gold (1974, 1976) has attempted to decrease the tumour-induced lactate recycling by inhibiting phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis. This was found to have only a limited effect on gluconeogenesis, since a block at this enzyme site inhibits the conversion of carbohydrate and protein to glucose, but has little effect on the synthesis of glucose from glycerol (Tisdale, 1982). Recycling of glucose may also occur from pyruvate and alanine, and gluconeogenesis from these substrates has also been found to be high in cancer patients (Waterhouse et al, 1979). Under normal circumstances pyruvate is oxidised to acetyl-CoA, which is irreversible and represents a loss to body carbohydrate reserves because animal cells lack the capacity for the synthesis of glucose from acetyl-CoA (Lehninger, 1975a). Pyruvate oxidation is inhibited by lowered insulin concentrations which allows the utilisation of pyruvate as well as lactate and alanine as substrates for gluconeogenesis. In the present study, little difference was found in the concentration of pyruvate between tumour-bearing and non tumour-bearing mice. However, this finding may be due to the fact that pyruvate decomposes very quickly and it is therefore difficult to measure levels of this metabolite accurately.

Levels of carnitine and glycerol were also measured, and although these were found to be higher in tumour-bearing mice compared to non tumour-bearing mice, these differences were not significant. The role of carnitine was discussed briefly in Section D.2.11, and the level of this metabolite reflects the rate of lipolysis which is high in the tumour-bearing state. Glycerol is a product of lipolysis and may be used as a precursor for gluconeogenesis (Lehninger, 1975b).

Amino acid levels were reduced in tumour-bearing animals with

the exception of taurine, aspartic acid and histidine. Protein serves as an additional reserve of metabolic fuel, which becomes depleted during the progressive wasting seen in cachexia (Jeevanandam et al, 1984). Free amino acids either from dietary or endogenous sources function both as gluconeogenic precursors and as a metabolically important form of nitrogen. Reduction of plasma amino acids in tumour-bearing animals is presumably therefore a reflection of an increased consumption of these amino acids by the tumour.

Levels of 3-hydroxybutyrate and acetoacetate were unaffected by the presence of the MAC 16 tumour. The lack of ketosis in tumour-bearing mice despite the loss of body fat and other components may be related to an elevated basal metabolic rate (Theologides, 1979) and an increase in energy expenditure by the liver due to increased Cori cycle activity. Attempts to regulate the metabolism of cancer patients in vivo are not new, the work of Gold (1974, 1976) has already been mentioned. Forced feeding regimens have also been used to overcome the problem of cachexia, but they have been associated with increased metabolic rates and increased tumour growth (Burke and Kark, 1977; Conyers et al, 1979a; Daly et al, 1980). In the present study, the effects of feeding low carbohydrate ketogenic diets, with increasing amounts of energy derived from medium chain triglyceride with and without 3-hydroxybutyrate arginine salt, were studied in NMR I mice implanted with the MAC 16 adenocarcinoma and in non tumour-bearing mice. The rationale for doing this was covered earlier.

Weight loss and tumour size were significantly reduced in mice fed a diet in which 80% of the energy was derived from medium chain triglyceride supplemented with 3-hydroxybutyrate arginine salt, compared to tumour-bearing mice fed on the normal diet. There were

also reductions in these parameters in the other groups fed ketogenic diets, but these were not significant when analysed statistically. A relationship was found between diet, the amount of weight loss and tumour size. These results show that it is possible to reduce weight loss and tumour size by feeding high fat diets supplemented with 3-hydroxybutyrate, and that the reduction is dependent upon the level of ketosis induced, which is, in turn, determined by the amount of fat in the diet. Waterhouse and Nye (1961) found that an increase in body weight occurred after infusion of triglyceride into patients with advanced cancer, and this was correlated with a gain of intracellular material. Buzby and associates (1980) demonstrated that it was possible to reduce tumour growth and restore nutritional reserves by using fat-based parenteral hyperalimentation in rats bearing a transplantable mammary adenocarcinoma. In studies using MCA-sarcoma-bearing rats, the survival of these animals was significantly increased by feeding diets high in fat and protein and low in carbohydrate (Demetrakopoulas and Rosenthal, 1982). Anorexia was prevented in rats bearing the Walker 256 carcinoma by feeding similar diets (Enrione et al, 1983). Magee et al (1979) found that dietary induced ketosis reduced the number of B16 melanoma deposits in the lungs of C57BL/b mice by two thirds.

Alterations in total carcass fat content were found in all the tumour-bearing groups of mice and in those non tumour-bearing groups of mice fed the high MCT diets. This is not surprising since it has been established both by earlier work in this study and in the literature, that this is a feature of cancer cachexia (Costa, 1977; Brennan, 1977; Watson et al, 1980). Loss of carcass fat was reduced in tumour-bearing mice by feeding high MCT ketogenic diets, presumably because of the direct inhibitory effect of ketone bodies

on lipolysis. The loss of carcass fat which occurred in the non tumour-bearing groups of mice fed the high MCT diets was not due to decreased dietary intake, but may be associated with the reduction in carbohydrate content of the diets. Supplementation of the normal diet with 20% sucrose resulted in a significant reduction ($p < 0.05$) in the loss of carcass fat from tumour-bearing mice compared to tumour-bearing mice fed the normal diet alone. Upon ingestion, sucrose is hydrolysed to glucose and fructose by sucrase, and studies have shown that fructose has a greater tendency to serve as a lipid precursor than glucose (Higgins, 1916; MacDonald et al, 1964; Nikkila et al, 1965; Kuo et al, 1967; Bar-On et al, 1968). Sucrose and fructose diets are known to stimulate hepatic lipogenesis and increase serum triglyceride and cholesterol levels, although part of this increase has been shown to result from increased lipolysis in adipose tissue (Shafrir, 1985). Thus, it is possible that the reduction in loss of carcass lipid from tumour-bearing mice fed a normal diet supplemented with sucrose, may be a direct result of the stimulatory effect of sucrose on lipogenesis and the provision of an additional source of lipid precursor in the form of fructose.

Blood levels of ketone bodies were not markedly elevated despite the high intake of triglyceride, presumably due to the high metabolic rate of the mice and the excretion of ketone bodies in the urine. Dietary modification had little effect on blood lactate and glucose levels and plasma insulin in tumour-bearing mice. There was a small reduction in plasma free fatty acid levels in animals on the high MCT diets possibly due to the direct anti-lipolytic effect of ketone bodies.

Alanine and glutamine are the predominant amino acids released from skeletal muscle (Pozetsky et al, 1969; Felig et al, 1970, 1973),

and alanine may be used as a substrate for gluconeogenesis (Waterhouse et al, 1968; Caldecourt et al, 1985). Sherwin et al (1975) found that the infusion of 3-hydroxybutyrate resulted in a specific decline in plasma alanine levels. In the present study, plasma alanine levels were reduced in animals fed the ketogenic diets, and this reduction may be a direct result of the ketosis induced. Ketone bodies have also been shown to inhibit the oxidation of branched-chain amino acids, leucine, isoleucine and valine (Buse et al, 1972). This can result in increased levels of these amino acids which in turn can lead to a stimulation of protein synthesis and an inhibition of protein degradation in muscle, particularly by leucine (Adibi, 1984). Branched-chain amino acids are also involved in the synthesis of alanine in skeletal muscle (Odessey et al, 1974). Inhibition of the oxidation of these amino acids, which is coupled to the synthesis of alanine, would therefore result in a reduction in alanine synthesis.

Levels of the branched-chain amino acids leucine, isoleucine and valine, were much higher in the groups of mice fed the ketogenic diets. This result suggests that the increased levels of branched-chain amino acids found in the mice fed the ketogenic diets may have arisen because of the inhibitory effects of elevated ketone body levels present in these groups on their oxidation and that this, in turn, could also contribute to the reduction in plasma alanine levels seen in these groups. The overall result would be a reduction in proteolysis from muscle, a reduction in gluconeogenesis and therefore conservation of body components.

Dietary modification also resulted in considerable alteration in histological structure of the MAC 16 adenocarcinoma. Under the normal dietary regime the tumour is poorly vascularised and shows

extensive necrosis (Figure D.3.36). After 24 days on the high MCT diets, the areas of necrosis are much reduced and the degree of vascularisation increased (Figures D.3.38, D.3.39, D.3.40). The number of macrophages and neutrophils (in 6 randomly selected fields of the microscope) were counted in sections of the tumours taken from each of the different diet treatment groups. There were significantly fewer ($p < 0.05$) macrophages present in sections of tumour from animals fed the 80% MCT diet when compared with tumours from animals fed the normal diet. The number of macrophages were also reduced in tumours from the other high MCT diet treatment groups although the change was not significant. This reduction in the number of macrophages may be associated with the reduction in necrosis seen in the tumours from animals fed the high MCT diets. Beutler et al (1985) recently reported the isolation of a macrophage derived protein which was first called cachectin because it was suspected of mediating cachexia in disease. Cachectin has been found to suppress the activity of the enzyme lipoprotein lipase both *in vitro* and *in vivo*. Suppression of this enzyme leads to a hypertriglyceridaemic state in the affected animals, and it has therefore been suggested that cachectin may contribute to the lipid and protein catabolism that ultimately reduces the host to the cachectic state. Furthermore, cachectin has been found to be identical to tumour necrosis factor (TNF), another macrophage product, and it has been speculated (Editorial, The Lancet, 1985) that TNF/cachectin can be induced by tumour cells. Aderka and co-workers (1985) examined the induction of TNF in peripheral blood mononuclear cells (PBMC) of patients with solid tumours, and found that spontaneous production of TNF occurred in a significant proportion of the cancer patients. This finding suggested that in cancer patients, continuous production

of cachectin/TNF may occur in response to sustained stimulation by tumour cells. The administration of cachectin to animals has been found to induce a state of anorexia with ensuing weight loss, coupled with an apathetic unkempt appearance reminiscent of cachexia observed under natural circumstances (Beutler and Cerami, 1986). A reduction in the number of macrophages would therefore be expected to result in a fall in the production of TNF/cachectin, and since TNF/cachectin is thought to be responsible for cachexia, this fall could be a contributory factor towards some of the effects seen in the mice fed high MCT diets in the present study, although to date TNF/cachectin has not been detected in serum from animals bearing the MAC 16 adenocarcinoma (Professor J Playfair, Middlesex Hospital, personal communication). It seems likely that factors such as Toxohormone L and TNF/cachectin may play major roles in the cachectic process and further investigation is necessary to try and identify factors similar to these in the MAC 16 tumour model. There were significantly more ($p < 0.05$) neutrophils present in sections of tumour from animals fed the 80% MCT diets compared to numbers present in tumours from the groups fed on the normal diet, and this increase may be associated with the increased vascularity of the tumours in this group.

Tumour architecture is an important determining factor in the effectiveness of antineoplastic therapy and the changes in morphological structure seen in tumours of mice fed the high MCT diets may, in fact, make the tumour more vulnerable to antineoplastic agents. The resistance of solid tumours such as the MAC 16 adenocarcinoma to chemotherapy and radiotherapy is associated with a poor blood supply to the tumour and accompanying hypoxia. In most experimental tumours of a size less than 0.5 cm^3, new blood vessels, which arise as a

result of the production of angiogenesis factor by tumour cells, are open throughout the tumour and there is no necrosis (Folkman, 1985). Beyond this size, there may be gradual compression of the capillaries due to changes in extravascular pressure (Warren, 1970) resulting in the eventual closure of the vessels and the cessation of blood flow and hence the supply of oxygen to the core of the tumour. This results in the central necrosis of the tumour and adjacent to the areas of necrosis, regions of cells which are hypoxic due to the decreased oxygen tension but still viable. One reason why tumours may fail to be controlled by radiation is the problem presented by these hypoxic cells (Denekemp, 1983).

Following the absorption of ionising radiation, highly reactive free radicals are formed, the interaction of which with biologically important molecules within the cell is energetic enough to break chemical bonds. The fate of these free radicals is greatly influenced by the presence of oxygen and studies of cells in tissue culture have shown that when cells are deprived of oxygen, the radiation dose required to give a particular level of effect increases by a factor of 2 to 3 (Steel, 1984). Thus an adequate supply of oxygen to all parts of the tumour is essential for radiotherapy to be effective in killing the rapidly proliferating cells of the tumour.

The resistance of poorly vascularised, hypoxic solid tumours to conventional chemotherapy may be attributed to the following: firstly, the reduced oxygen tension may directly cause resistance and, secondly, the deficiency of oxygen and nutrients which exists in the hypoxic regions of the tumour may result in changes in the cell cycle of cells in these regions, causing them to become non-cycling or slowly cycling. This would confer resistance to cycle specific

drugs (Kennedy et al, 1980; Workman, 1983). Finally, resistance may arise simply because adequate supplies of chemotherapeutic agents are unable to reach the regions of hypoxic cells due to the poor vascularity of the tumour. An increase in vascularisation of the tumour and therefore in the supply of oxygen might be expected to lead to a reduction in hypoxia and the resistance to antineoplastic therapy.

This study has shown that it is possible to reduce host weight loss and tumour size by increasing the lipid contribution to the diet. In addition, such dietary modification may have an important synergistic action with conventional chemotherapy and radiotherapy. In a recently completed study (Fearon et al, 1986), systemic ketosis was induced in five patients with advanced malignant disease and severe weight loss by the infusion of 70% medium chain triglyceride supplemented with 3-hydroxybutyrate arginine salt. Such a regime was found to be well tolerated and caused a weight gain in all patients. Another study in which normal human subjects were fed a diet in which 85% of the calories were supplied from fat, was also found to be well tolerated (Phinney et al, 1983). Subjects were given a choice from a menu which included meals prepared from ground beef, cheddar cheese, soured cream and tuna fish, thus demonstrating that it would be possible to draw up a high fat ketogenic diet from everyday food items. In addition to the diet being well tolerated, there was no measurable impairment of hepatic, renal, cardiac or haemopoietic function. The present study is one of the first examples of an attempt to reverse cachexia using dietary means based on biochemical differences between host and tumour tissues which aims to selectively feed the host at the expense of the tumour.

F. Appendix

G. References

References

Adibi S A (1984). Nutritional, physiological and clinical significance of branched chain amino acids. In: Branched chain amino and keto acids in health and disease. Eds. Adibi S A, Fekl W, Langenbeck U, Schauder P. Karger, Basel. pp1-14.

Ali S A, Bibby M C, Double J A (1985). Body weight loss (cancer cachexia) following transplantation of an adenocarcinoma of the mouse colon (MAC 16). Br. J. Cancer, 52, p452.

Aderka D, Fisher S, Levo Y, Holtmann H, Hahn T, Wallach D (1985). Cachectin/tumour necrosis factor production by cancer patients. The Lancet, 2, p1190.

Bar-On H, Stein J (1968). Effect of glucose and fructose administration on lipid metabolism in the rat. J. Nutr., 94, pp95-105.

Bates M W (1972). Effects of hydroxybutyrate infusion and insulin injection on ketone body turnover of rats. Amr. J. Physiol., 222, pp462-467.

Bates M W, Krebs H A, Williamson D H (1968). Turnover rates of ketone bodies in normal, starved and alloxan-diabetic rats. Biochem. J., 110, pp655-661.

Beutler B, Mahoney J, Le Trang N, Pekala P, Cerami A (1985a). Purification of cachectin, a lipoprotein lipase suppressing hormone secreted by endotoxin induced RAW 264.7 cells. J. Exp. Med., 161, pp981-995.

Beutler B, Greenwald D, Holmes J D, Chang M, Pan Y C E, Mathison J, Ulevitch R, Cerami A (1985b). Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. Nature, 316, pp552-554.

Beutler B, Cerami A (1986). Cachectin and tumour-necrosis factor as two sides of the same biological coin. Nature, 320, pp584-588.

Blazquez E, Lopez-Quijada C (1969). The effect of high carbohydrate diet on glucose insulin sensitivity and plasma insulin in rats. J. Endocrinology, 44, pp107-113.

Bloch-Frankenthal L, Langan J, Morris H P, Weinhouse S (1965). Fatty acid oxidation and ketogenesis in transplantable liver tumours. Can. Res., 25, pp732-736.

Bozzetti F (1984). Malnutrition in cancer and cachectic patients. In: Marker proteins in inflammation, Vol 2. Ed. P Arnold. Walter de Gruyter & Co, Berlin, New York. pp465-476.

Brennan M F (1977). Uncomplicated starvation versus cancer cachexia. Can. Res., 37, pp2359-2364.

Burke M, Kark A E (1977). Parenteral feeding and cancer. The Lancet, 1, p999.

Buse M G, Biggers J F, Friderici K H, Buse J F (1972). Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat. The effect of fatty acids, glucose and pyruvate respiration. J. Biol. Chem., 247, pp8085-8096.

Butcher R W (1968). Role of cyclic AMP in hormone actions. N. Eng. J. Med., 279, pp1378-1384.

Buzby G P, Mullen J L, Stein T P, Miller E E, Hobbs C L, Rosato E F (1980). Host-tumor interaction and nutrient supply. Cancer, 48, pp2940-2948.

Cahill G F (1970). Starvation in man. New Engl. J. Med., 282, pp668-675.

Caldecourt M A, Cox D J, Sugden M C, Palmer T N (1985). Glycolytic origin of alanine formed in rat diaphragm muscle in vitro. Biochem. J., 231, pp801-804.

Chance W T, Van Lammeran F M, Chen M H, Murphy R F, Joffe S N, Fischer J E (1983). Alteration in plasma levels of insulin and glucagon associated with cancer anorexia. Surg. Forum, 34, pp441-443.

Clark C M, Goodland G A J (1971). Depletion of protein of phasic and tonic muscles in tumor-bearing rats. Eur. J. Can., 7, pp3-9.

Conyers R A J, Need A G, Durbridge T, Harvey N D M, Potezny N, Rofe A M (1979a). Cancer, ketosis and parenteral nutrition. Med. J. Aust., 1, pp398-399.

Conyers R A J, Need A G, Rofe A M, Potenzy N, Kimber R J (1979b). Nutrition and cancer. Br. Med. J. 1, p1146.

Costa G, Holland J F (1962). Effects of Krebs-2 carcinoma on the lipide metabolism of male swiss mice. Can. Res., 22, pp1081-1083.

Costa G (1977). Cachexia, the metabolic component of neoplastic diseases. Can. Res., 37, pp2327-2335.

Copeland E M, Rodman C A, Dudrick S J (1979). Nutritional concepts of neoplastic disease. In: Nutrition and Cancer. Eds: van Eys J, Seelig M S, Nichols B L. Spectrum, New York, pp133-156.

Czok R, Lamprecht W (1974). Pyruvate, phosphoenolpyruvate and D-glycerate-2-phosphate. In: Methods of enzymatic analysis, Vol 3. Ed: Bergmeyer H U. Academic Press, pp1446-1451.

Daly J M, Reynolds H M, Rowlands B J, Dudrick S J, Copeland E M (1980). Tumor growth in experimental animals: nutritional manipulation and chemotherapeutic response in the rat. Ann. Surg., 191, pp316-322.

Denekemp J (1983). Does physiological hypoxia matter in cancer chemotherapy? In: Biological basis of radiotherapy. Eds: Steel G G, Adams G E, Peckham M J. Elsevier, Amsterdam, pp139-155.

Demetrakopoulas G, Rosenthall A (1982). Prolonged survival of MCA-sarcoma bearing rats fed with a low carbohydrate diet. Proc. Am. Assoc. Cancer Res., 23, p10.

Devereux D F, Roby C, Deckers P J, Pietrafitta J, Mozden P J (1982). Effect of tumor bearing and removal on lipid metabolism in the rat. Surg. Forum, 38, pp406-408.

Double J A, Ball C R, Cowen P N (1975). Brief communication: Transplantation of adenocarcinoma of the colon in mice. JNCI, 54, 271-275.

Editorial (1985). Cachectin. The Lancet, 2, pp312-313.

Enrione E B, Black C D, Morre D M (1983). High fat total parenteral nutrition in tumor-bearing rats. Fed. Proc., 42, p1070.

Fearon K C H, Tisdale M J, Preston T, Plumb J A, Calman K C (1985). Failure of systemic ketosis to control cachexia and the growth rate of the Walker 256 carcinosarcoma in rats. Br. J. Cancer, 52, pp87-92.

Fearon K C H, Borland W, Preston T, Tisdale M J, Shenkin A, Calman K C (1986). Influence of systemic ketosis on substrate levels and nitrogen metabolism in cancer cachexia. (Submitted for publication).

Felig P, Pozefsky T, Marliss E, Cahill G F (1970). Alanine: Key role in gluconeogenesis. Science, 167, pp1003-1004.

Felig P, Wahren J, Raf L (1973). Evidence of inter-organ amino acid transport by blood cells in humans. Proc. Natl. Acad. Sci. USA, 70, pp1775-1779.

Fenninger L D, Mider G B (1954). Energy and nitrogen metabolism in cancer. Adv. Cancer Res., 2, pp225-229.

Fenselau A, Wallis K (1974). Comparative studies of 3-oxo acid-Coenzyme A transferase from various rat tissues. Biochem. J. 142, pp619-627.

Feteris W A (1965). A serum glucose method without protein precipitation. Am. J. Med. Technol., 31, pp17-21.

Fields A L A, Walman S L, Cheema-Dhadi S, Morris H P, Halperin M L (1981). Regulation of energy metabolism in Morris Hepatoma 7777 and 7800. Can. Res. 41, pp2762-2766.

Folkman J (1985). Tumor angiogenesis. Adv. Can. Res., 43, pp175-203.

Frederick G L, Begg R W (1956). A study of hyperlipidemia in the tumor-bearing rat. Can. Res., 16, pp548-558.

Fredericks M, Ramsey R B (1978). 3-oxo acid-Coenzyme A transferase activity in brain and tumours of the nervous system. J. Neurochem., 31, pp1529-1531.

Gold J (1966). Metabolic profiles in human solid tumors. 1. A new technic for the utilisation of human solid tumors in cancer research and application to the anaerobic glycolysis of isologous benign and malignant colon tissues. Can. Res., 26, pp695-705.

Gold J (1974). Cancer cachexia and gluconeogenesis. Annals. New York Acad. Sci., 230, pp103-111.

Gold J (1976). Hydrazine sulphate in the treatment of cancer. Can. Treat. Reps., 60, pp964-965.

Greene H L, Hulsey T, Hoffman L H, Bennett S (1979). The effects of malnutrition and cancer therapy agents on small bowel. In: Nutrition and Cancer, Eds: Van Eys J, Seelig M S, Nichols B L. Spectrum, New York, pp71-90.

Gutman I, Wahlefield A W (1974). L-(+)-Lactate determination with lactate dehydrogenase and NAD. In: Methods of enzymatic analysis. Vol 3. Ed: Bergmeyer H U. Academic Press, pp1464-1468.

Harnet N L (1952). British Empire Cancer Campaign. A survey of cancer in London. British Empire Cancer Campaign, p26.

Hawkins R A, Alberti K G M M, Houghton C R S, Williamson D H, Krebs H A (1971). The effect of acetoacetate on plasma insulin concentration. Biochem. J., 25, pp541-544.

Higgins H L (1916). The rapidity with which alcohol and some sugars may serve as nutriment. Am. J. Physiol., 41, pp258-263.

Holroyde C P, Gabuzda T G, Putnam R C, Ponul A, Reichard G A (1975). Altered glucose metabolism in metastatic carcinoma. Can. Res., 35, pp3710-3714.

Holroyde C P, Axelrod R S, Skutches C L, Haff A C, Paul P, Reichard G A (1979). Lactate metabolism in patients with metastatic colorectal cancer. Can. Res., 39, pp4900-4904.

Hyvarinen A, Nikkila E (1962). Specific determination of blood glucose with O-toluidine. Clin. Chem. Acta, 7, pp140-143.

Inagaki J, Rodriguez V, Bodey G B (1974). Proceedings: Causes of death in cancer patients. Cancer, 33, pp568-573.

Jeevanandam M, Horowitz G D, Lowry S F, Brennan M F (1984). Cancer cachexia and protein metabolism. The Lancet, 1, pp1423-1426.

Kennedy K A, Teicher B A, Rockwell S, Sartorelli A C (1980). The hypoxic tumor cell: A target for selective cancer chemotherapy. Biochem. Pharmacol., 29, pp1-8.

Kitada S, Hays E F, Mead J F (1980). A lipid mobilising factor in serum of tumor-bearing mice. Lipids, 15, pp168-174.

Kralovic R C, Zepp E A, Cenedella R J (1977). Studies of the mechanism of carcass fat depletion in experimental cancer. Europ. J. Cancer, 13, pp1071-1079.

Kuo P T, Feng L, Cohen N N, Fitts W T, Miller L D (1967). Dietary carbohydrate in hyperlipemia (hypertriglyceridemia) hepatic and adipose tissue lipogenic activities. Am. J. Clin. Nutr., 20, pp116-125.

Lanza-Jacoby S, Miller E E, Rosato F E (1982). Changes in the activities of lipoprotein lipase and the lipogenic enzymes in tumor-bearing rats. Lipids, 17, pp944-949.

Lazo P A (1985). Tumor-host metabolic interaction and cachexia. FEBS, 187, pp189-192.

Lehninger A L (1975a). Biochemistry. 2nd edition. Worth Publishers Inc, New York, p630.

Lehninger A L (1975b). Biochemistry. 2nd edition. Worth Publishers Inc, New York, p842.

Lowry O H, Rosebrough N J, Farr A L, Randall R (1951). Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193, pp255-275.

Lundholm K, Edstrom S, Karlberg I, Ekman L, Schersten T (1980). Relationship of food intake, body composition and tumor growth to host metabolism in nongrowing mice with sarcoma. Can. Res., 40, pp2516-2522.

Macdonald I, Braithwaite D M (1964). The influence of dietary carbohydrates on the lipid pattern in serum and in adipose tissue. Clin. Sci., 27, pp23-30.

Magee B A, Potenzny N, Rofe A M, Conyers R A J (1979). The inhibition of malignant cell growth by ketone bodies. Aust. J. Exp. Biol. and Med. Sci., 57, pp529-549.

Masuno H, Yamasaki N, Okuda H (1981). Purification and characterisation of a lipolytic factor (toxohormone-L) from cell-free fluid of ascites Sarcoma 180. Can. Res., 41, pp284-288.

Mays E T (1969). Serum lipids in human cancer. J. Surg. Res., 9, pp273-277.

Mellanby J, Williamson D (1974). Acetoacetate. In: Methods of enzymatic analysis. Vol 4. Ed: Bergmeyer H U. Academic Press, pp1840-1843.

Mermier P, Baker N (1974). Flux of free fatty acids among host tissues, ascites fluid and Ehrlich ascites carcinoma cells. J. Lipid Res., 15, pp339-351.

Mider G B, Sherman C D, Morton J J (1949). The effect of Walker carcinoma 256 on the total lipid content of rats. Can. Res., 9, pp222-224.

Moley J F, Morrison S D, Norton J A (1985). Insulin reversal of cancer cachexia in rats. Can. Res., 45, pp4925-4931.

Morrison S D (1979). Anorexia and the cancer patient. In: Nutrition and cancer. Eds: Van Eys J, Seelig M S, Nichols B L. Spectrum, New York, pp31-47.

Nikkila E A, Oyada K (1965). Induction of hypertriglyceridemia by fructose in the rat. Life Sci., 4, pp937-943.

Nixon D W, Lawson D H, Kutner M, Ansley J, Schwarz M (1981). Hyperalimentation of the cancer patient with protein-calorie undernutrition. Can. Res., 41, pp2038-2045.

Odessey R, Khairallah E A, Goldberg A L (1974). Origin and possible significance of alanine production by skeletal muscle. J. Biol. Chem., 249, pp7623-7629.

Ookhtens M, Baker N (1979). Evaluation of impaired triglyceride fatty acid transport and oxidation for the detection of cancer in mice. Can. Res., 39, pp5118-5123.

Owen O E, Morgan A P, Kemp H G, Sullivan J M, Herrera M G, Cahill G F (1967). Brain metabolism during fasting. J. Clin. Invest., 46, pp1589-1595.

Pearson D J, Tubbs P K, Chase J F A (1974). L-Carnitine. In: Methods of enzymatic analysis. Vol 4. Ed: Bergmeyer H U. Academic Press, pp1758-1762.

Phinney S D, Bistrian B R, Wolfe R R, Blackburn G L (1983). The human metabolic response to chronic ketosis without caloric restriction. Physical and biochemical adaptation. Metabolism, 32, pp757-768.

Pozefsky T, Felig P, Tobin J D, Soeldner J S, Cahill G F (1969). Amino acid balance across tissues of the forearm in postabsorptive man. Effects of insulin at two dose levels. J. Clin. Invest. 48, pp2273-2282.

Redgrave T G, Devereux D F, Deckers P J (1984). Hyperlipidemia in tumor-bearing rats. Biochim. et Biophys. Acta, 795, pp286-292.

Robinson A M, Williamson, D H (1980). Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol. Rev., 60, pp143-187.

Schien P S, Kinner D, Haller D, Blecher M, Hamosh M (1979). Cachexia of malignancy: Potential role of insulin in nutritional management. Cancer, 43, pp2070-2076.

Shafrir E (1985). Effect of sucrose and fructose on carbohydrate metabolism and the resulting consequences. In: Regulation of carbohydrate metabolism. Vol 2. Ed: Beitner R. CRC Press, Ohio, USA, pp95-140.

Sherwin R S, Hendler R G, Felig P (1975). Effect of ketone infusions on amino acid and nitrogen metabolism in man. J. Clin. Invest., 55, pp1382-1390.

Steel G G (1984). New light on the radiosensitivity of human tumour cells. Cancer Topics, 5, pp4-5.

Stein T P (1978). Cachexia, gluconeogenesis and progressive weight loss in cancer patients. J. Theor. Biol., 73, pp51-59.

Stern J R, Coon M J, del Campillo A, Schneider M C (1956). Enzymes of fatty acid metabolism. IV. Preparation and properties of coenzyme A transferase. J. Biol. Chem., 221, pp15-31.

Strain A J, Easty G C, Neville A Munro (1980). An experimental model of cachexia induced by a xenografted human tumour. JNCI, 64, pp217-221.

Theologides A (1979). Cancer cachexia. Cancer, 43, pp2004-2012.

Tisdale M J (1982). Tumour and host nutrition. Cancer Topics, 3, pp113-115.

Tisdale M J, Brennan R A (1983). Loss of acetoacetate coenzyme A transferase activity in tumours of peripheral tissues. Br. J. Cancer, 47, pp293-297.

Tisdale M J, Brennan R A (1986). Metabolic substrate utilisation by a tumour cell line which induces cachexia in vivo. Br. J. Cancer, 54.

Warburg O (1930). In: The metabolism of tumours. Constable and Co, London.

Warnold I, Lundholm K, Schersten T (1978). Energy balance and body composition in cancer patients. Can. Res., 38, pp1801-1807.

Warren B A (1970). The ultrastructure of the microcirculation at the advancing edge of Walker 256 carcinoma. Microvasc. Res., 2, pp443-453.

Waterhouse C, Nye W H R (1961). Metabolic effects of infused triglyceride. Metabolism, 10, pp404-414.

Waterhouse C (1974). How tumours affect host metabolism. Ann. NY Acad. Sci., 230, pp86-73.

Waterhouse C, Jeanpetre N, Keilson J (1979). Gluconeogenesis from alanine in patients with progressive malignant disease. Can. Res. 39, pp1968-1972.

Watson W S, Sammon A M (1980). Body composition in cachexia resulting from malignant and non-malignant diseases. Cancer, 46, pp2041-2046.

Watkin D M (1959). Increased fat utilisation in the hypermetabolism of active neoplastic disease. Acta Un. Int. Cancer, 15, pp907-917.

Wieland O (1974). Glycerol. U-V method. In: Methods of enzymatic analysis. Vol 3. Ed: Bergmeyer H U. Academic Press, pp1404-1409.

Williams J F, Matthaei K I (1981). Cancer induced body wasting. A review of cancer cachexia and a hypothesis concerning the molecular basis of the condition. Asean J. Clin. Sci., 2, pp158-167.

Williamson D H, Bates M W, Page M A, Krebs H A (1971). Activities of enzymes involved in acetoacetate utilisation in adult mammalian tissues. Biochem. J., 121, pp41-47.

Williamson D H, Mellanby J (1974). D-(-)3-Hydroxybutyrate. In: Methods of enzymatic analysis. Vol 4. Ed: Bergmeyer H U. Academic Press, pp1836-1839.

Wiseman G, Ghadially F N (1955). Studies in amino acid uptake by RD3 sarcoma cell suspensions in vitro. Br. J. Can., 9, pp480-485.

Workman P (1983). New drugs against hypoxic cells (Part II). Cancer Topics, 4, pp54-57.